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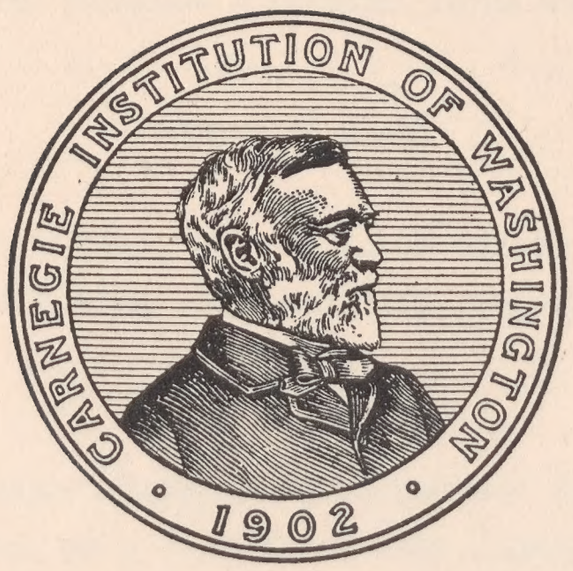


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CONTRIBUTIONS TO EMBRYOLOGY, NO. 207

# ALKALINE PHOSPHATASE IN THE OVARIAN FOLLICLE AND IN THE CORPUS LUTEUM

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With five plates

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# ALKALINE PHOSPHATASE IN THE OVARIAN FOLLICLE AND IN THE CORPUS LUTEUM

## INTRODUCTION

For more than one hundred years histologists have been discussing the origin of the corpus luteum and seeking to trace the respective roles of the membrana granulosa and theca interna of the ovarian follicle, when the follicle is converted into the corpus luteum. Two major problems have presented themselves. The first of these, which concerns the role of the granulosa, may be regarded as solved. In all mammals thus far studied the cells of the granulosa layer persist after discharge and collapse of the follicle and are converted into "luteal" cells, forming the greater part of the substance of the corpus luteum.

The second question concerns the fate of the theca interna. On this there is as yet no complete agreement, and it appears that the situation is not alike in all species. Some writers have described the conversion of the theca cells into the connective-tissue framework of the corpus luteum; others have reported that the theca cells persist in the fully formed corpus luteum and that they can be distinguished from the granulosa luteal cells. It seems clear, for example, that in the human corpus luteum the theca interna remains in its original position in relation to the other layers of the follicle wall, and thus it is present in the corpus luteum as a special layer ("thecal gland") about the periphery of the corpus luteum and in the septa that are produced by the folding of the follicle wall at the time of its collapse. In the domestic pig, on the contrary, the present author described (1919) the inwandering and dispersal of part of the theca cells and their persistence as scattered elements among the granulosa luteal cells. New evidence for this interpretation, which when originally published was promptly contradicted (Solomons and Gatenby, 1924), will form the major part of the present paper. For references to the extensive literature and for a more detailed account of the subject, the reader may consult two previously published articles by Corner (1919, 1945).

This question of the origin of the corpus luteum is one of those problems, well known to students of morphogenesis, which seem easy in prospect and yet elude solution because the cell types involved are not sufficiently distinctive to be followed clearly as the tissues change and cells shift their position and modify their characteristics. So far as we know, the theca cells and the granulosa cells have a common ancestry in the cells of the ovarian mesoblast. They happen to be distinguishable in the follicle by reason of differences in situation, in staining characteristics of nuclei and cytoplasm, in proportion of cytoplasm to nucleus, and in cytoplasmic lipid inclusions; but all these characteristics can and do change during the rapid period of conversion from follicle to corpus luteum.

No doubt every investigator who has worked on this difficult problem has dreamed of a selective stain or microchemical agent that would distinguish the cells of the theca interna sharply from those of the membrana granulosa of the mature ovarian follicle and of the early corpus luteum. Such a means of detection has unexpectedly presented itself for trial, through the invention by Gomori (1939) of a microchemical test for the presence of an enzyme, alkaline phosphatase, which is capable of freeing phosphate ions from glycerophosphate in an alkaline medium.

Gomori's first general exploration of animal tissues with his new method (1941) revealed that in the ovaries of various animals the distribution of the enzyme is related to the histological structure. It showed, moreover, the presence of two types of cells in the sow's corpus luteum. The present writer undertook, therefore, a more detailed study of the distribution of phosphatase, with particular reference to the theca interna and the granulosa cells of the follicle and their derivatives in the corpus luteum.

## METHOD

Gomori's method depends upon the fact that the action of alkaline phosphatase is not affected by fixation in 80 per cent alcohol and subsequent complete dehydration and embedding in paraffin. Sections on the slide are incubated in a buffered solution of sodium glycerophosphate containing calcium chloride. The phosphate ions freed by action of the enzyme unite at once with calcium ions, forming insoluble

calcium phosphate. The slides are then passed in succession through solutions of cobalt chloride and of ammonium sulphide. The calcium phosphate is thus converted first into cobalt phosphate and ultimately into cobalt sulphide, an insoluble black compound which is readily visible under the microscope. All the author's preparations described in the present article were made according to Gomori's directions of 1941, except that the period of incubation was lengthened to 2 or 2½ hours instead of 1½ hours. Control preparations were made by omitting the incubation in the glycerophosphate solution, in order to detect false reactions due to preformed deposits of calcium phosphate. Such deposits were not found in any of the ovaries studied.

All surgical operations were done under anesthesia.

## OBSERVATIONS ON OVARIES OF THE PIG

Preparations were made from the ovaries of 17 sows selected to cover the successive stages from the maturing follicle (5 mm. diameter) through the mature follicle, the recently ruptured follicle, the incipient and the fully formed corpus luteum up to the age of about 10 days. These specimens were fixed at the slaughterhouse immediately after removal of the viscera. They were arranged in a series and dated with respect to the ovarian cycle, by inspection of the ovaries, uteri, ova, and embryos, and (when necessary) by microscopic examination of the ovaries and uteri, according to the descriptions of Corner (1921). A brief account of this method of dating sows' ovaries so as to follow the cycle in slaughterhouse material is given by Kimura and Cornwell (1938).

### *The Follicle*

Only casual observations were made on alkaline phosphatase in early and maturing follicles. Figure 1, plate 1, serves to illustrate a fact which must be mentioned at the beginning, which is that the nuclei of practically all the cells contain alkaline phosphatase. The great differences between the phosphatase content of some of the nuclei and that of others (e.g. the granulosa and theca interna cells in the follicle illustrated, as compared with the nuclei of stroma cells outside the follicle) may be related to metabolic condition and are probably worthy of special study. We are concerned in this report, however, with the cytoplasm only. In very young follicles, as yet having no antra, the spindle-shaped theca interna cells contain no cytoplasmic phosphatase.

As shown in figure 2, plate 1, the theca interna of a relatively small follicle, about 0.5 mm. in average diameter, contains a fairly rich deposit of phosphatase, indicated by the black zone just outside the granulosa. The same is true of a much larger follicle shown in the same picture, which is 5 mm. in diameter and was presumably entering upon the final stage of growth preparatory to ovulation a few days later.

The theca interna cells of the mature follicle contain heavy deposits of phosphatase in the cytoplasm. In contrast, the granulosa cells contain the enzyme only in the nuclei. These facts are clearly illustrated in the photograph of a 10-mm. follicle about 1 day before ovulation (fig. 3, pl. 1).

### *Early Corpus Luteum*

The sharp difference between granulosa and theca interna, with respect to their phosphatase content, is strikingly shown in figure 4, plate 1, which represents a corpus luteum about 1 day after ovulation (day 2 of the cycle). This follicle happened to collapse, after rupture, into an unusually complex system of folds, which are outlined by the thin zone of blackened theca interna lying everywhere between the folded granulosa and theca externa. At higher magnification (fig. 5, pl. 1) the theca interna is seen to be as yet distinct from the granulosa, except at the tips of the folds, where the first ingrowth of capillaries from the theca interna begins.

On the 3d or 4th day of the cycle (figs. 6, 7, pl. 2) the process of vascularization is well under way. Capillaries have penetrated among the granulosa cells. With high magnification (fig. 7) it is clearly evident that some of the theca interna cells have become displaced from the original thecal zone and are located among the now enlarging granulosa cells, along the invading capillaries. A slightly more advanced stage

of this process (day 6 or 7 of the cycle) is shown in figure 8, plate 2. Since this immigration of the theca interna cells begins in the basal region of the granulosa, low-power views at the 6th to the 8th day after ovulation may show a zone next the former lumen which is lighter in color than the basal half of the granulosa, because the blackened theca interna cells have not penetrated it fully (fig. 9, pl. 2). The extent to which the commingling of theca interna cells and granulosa ultimately proceeds is strikingly illustrated by figures 10 and 11, plate 3, from a corpus luteum of a sow 18 or 19 days pregnant. The low-power photograph tends indeed to exaggerate the apparent number of scattered theca cells, because endothelial cells lining the blood capillaries also contain deposits of phosphatase, which apparently become denser as the corpus luteum grows older. The phosphatase-containing theca cells form only a part, therefore, of the numerous dark patches seen throughout the corpus luteum in such a preparation as that illustrated by figure 10. At high magnifications, however, there is no difficulty in distinguishing the cell types and it is quite obvious that epithelioid cells of theca interna type are present, singly and in small clumps along the capillaries throughout the substance of the corpus luteum. It must be emphasized that in order to produce such dispersion of theca cells throughout the corpus luteum, no individual cell need move farther than a distance corresponding to the thickness of the granulosa, after it is converted to luteal tissue.

As pregnancy advances, phosphatase begins to be deposited in the large granulosa luteal cells. This is already apparent in the ovaries of an animal bearing embryos of 46 mm. crown-rump length (about 5 weeks pregnant). At about 7 weeks (fetuses of 75 mm. crown-rump length) there is definite blackening of some of the granulosa cells, so that it begins to be difficult to distinguish the two cell types with certainty (fig. 12, pl. 3).

To summarize these observations, Gomori's test for alkaline phosphatase, when applied to the ovaries of swine, affords a reliable means of distinguishing the theca interna cells, which contain the enzyme, from the granulosa cells, which are devoid of it. This difference persists after ovulation and during the first few weeks of pregnancy. The preparations show that the epithelioid cells of the theca interna remain after ovulation as epithelioid cells in the corpus luteum. Some of them remain at the periphery of the corpus luteum in the original position of the theca interna, whereas others become scattered among the granulosa luteal cells. This confirms the description of Corner (1919), and on the other hand does not accord with the report of Solomons and Gatenby (1924), who denied the dispersal of theca cells among the granulosa in the corpus luteum of the sow.

## OBSERVATIONS ON OTHER ANIMALS

The observations on other species, next to be reported, were made on small numbers of specimens, 2 to 5 for each species. Conclusions from them should therefore be drawn cautiously. Gomori's method is, however, remarkably simple, constant, and uniform in its results, as pointed out by its inventor. Individual stages in the comparatively large series of pig ovaries were moreover highly consistent with one another. For these reasons it is believed that a few specimens from a given species, if properly prepared and chosen to represent significant stages, are worth reporting, if only as a stimulus to further study.

### *Rabbit*

Dated specimens of mature follicles and very early corpora lutea were obtained from 5 rabbits by the method usually employed with this species; that is, the females were mated in order to bring about ovulation 10 hours later. Figure 13, plate 3, representing a follicle of a rabbit killed 9 hours after coitus, shows that the granulosa cells contain moderately dense black deposits indicating the presence of phosphatase, whereas the theca interna is practically free of phosphatase. This is just the reverse of the situation in the domestic pig. In the ruptured follicle (early corpus luteum) 18 hours after mating, or about 10 hours after ovulation, the two layers are still distinguishable and the staining is the same. At 3 days after ovulation (fig. 14, pl. 4) the black deposits are so dense in the granulosa luteal cells that the theca cells, even if still without cytoplasmic phosphatase, could not surely be distinguished. Here and there a few clumps of clear cells may perhaps be identifiable as theca cells (fig. 14, *th. c.?*), but it would be impossible to identify any such cells that might have

wandered among the granulosa. Gomori's method, therefore, does not aid in determining the fate of the theca interna in the rabbit.

### *Guinea Pig*

Five animals were dated by observing the vaginal closure membrane. In almost mature follicles, 0.8 mm. in diameter, 1½ hours after opening of the membrane, there was a light deposit of phosphatase in the granulosa, stronger in the basal part of the layer. In the cells of the theca interna there was a heavy deposit (fig. 15, pl. 4). Three days after opening of the vaginal closure membrane, the early corpora lutea showed black deposits in all the epithelioid cells (fig. 16, pl. 4). It was therefore not possible to distinguish the theca interna cells from the granulosa derivatives. At later stages, for example 9 days after opening of the membrane, and in pregnancy with fetuses 24 mm. crown-rump length, there was a general moderately heavy deposit in the cells of the corpora lutea. Dr. Walter L. Hard gave the writer a large collection of pregnant guinea pigs' ovaries fixed in 80 per cent alcohol which have been sectioned and stained for phosphatase. From these it appears that the enzyme is abundantly present in the corpus luteum throughout pregnancy.

### *Dog*

Three bitches were studied. No. 1 was spayed 7 days after the onset of estrous bleeding. The ovaries contained unruptured follicles 3.5 mm. in diameter. There was a moderately heavy deposit of phosphatase in the epithelioid cells of the theca interna. The cytoplasm of the granulosa cells was faintly gray, indicating small amounts of phosphatase (figs. 17, 18, pl. 4). No. 3 was spayed 10 days after the onset of estrous bleeding. The ovaries contained corpora lutea estimated (by comparison with dated corpora lutea of sow and rhesus monkey) as 4 to 5 days old. A few epithelioid cells of the theca interna contained light deposits of phosphatase, but most of them were free of it. The theca interna of closely adjacent small and medium-sized follicles contained rich deposits (fig. 19, pl. 5). No. 2 was spayed 12 days after onset of estrous bleeding. A single coitus occurred 2 days before the operation. The ovaries contained corpora lutea estimated as 5 to 6 days old. There were no black deposits anywhere in the corpus luteum, except in small blood vessels.

It must be concluded that in the dog either the theca interna cells lose their identity, or, what is more likely, they give up their phosphatase within a few days after ovulation.

### *Rhesus Monkey*

Two monkeys were used for phosphatase study. An ovary of one of these was removed within 25 hours after ovulation, as determined by palpation of the ovaries. The phosphatase stain showed heavy deposits of the enzyme in both granulosa and theca interna of the recently ruptured follicle (fig. 20, pl. 5).

The other monkey was subjected to laparotomy and the ovary was removed between 8 and 11 days after ovulation. The black deposit, indicating phosphatase, was present in all the epithelioid cells of the corpus luteum. Theca interna cells were not distinguishable with certainty.

In this species, the follicles are evidently rich in phosphatase from an early stage of their development. Phosphatase is present in the theca interna of medium-sized follicles, both normal and atretic. Even the single layer of cells surrounding primordial ova is usually heavily laden with the enzyme. Part or all of the zona pellucida of the ovum is always stained. As a result, such a preparation as that shown in figure 20 gives a striking picture of the general pattern of the ovary.

### *Human*

Dr. Arthur T. Hertig very kindly prepared 3 human corpora lutea in the laboratories of the Free Hospital for Women, Brookline, Massachusetts (clinic of Dr. John Rock). Gomori's method of 1941 was followed exactly. The first (S-44-13) accompanied an endometrium in the late proliferative stage, with some early basal vacuolization, estimated by Dr. Hertig to be of the 16th day of the cycle and 2 days after ovulation. The writer, before learning the history, considered the corpus luteum to represent probably the first day after ovulation. The phosphatase stain was quite successful and the cells of the theca interna contained deposits of black pigment representing phosphatase. The granulosa cells contained no phosphatase. This corpus luteum closely resembled comparable stages in the domestic pig (fig. 21, pl. 5).

The second specimen (S-44-46) came from a patient whose endometrium was considered by Dr. Hertig to be of the 19-day type (calculated from onset of the last menstrual period). The corpus luteum was about 5 days old. The phosphatase reaction was positive in the endothelium and in many nuclei, but the cytoplasm of the theca interna cells (which were fully distinguishable because of their number and position, as is characteristic of the human species) gave no evidence of phosphatase. The granulosa was also unstained.

The third corpus luteum (S-44-37) accompanied a 7½-day embryo of the Hertig-Rock series, no. 8225 of the Carnegie Collection. The phosphatase reaction was positive in some endothelial cells and some nuclei, but the cells of the theca interna and of the granulosa were free of signs of phosphatase. A few cells of the granulosa near the inner surface contained light deposits of the enzyme (fig. 22, pl. 5).

There should of course be much more extensive study of human material. These specimens, as far as they go, indicate that the cells of the theca interna contain phosphatase up to a day or two after ovulation but subsequently lose the enzyme. The granulosa seems to be almost or quite free of it, in the stages examined. In these respects the human species resembles the dog more than it does the rhesus monkey.

## SUMMARY AND CONCLUSIONS

The diversity of ovarian structure in various mammals, a fact well known to students of the ovary, is again emphasized by the present observations. Six species have been studied and four different conditions noted, as shown in the accompanying table.

ALKALINE PHOSPHATASE IN MATURE OVARIAN FOLLICLES AND EARLY CORPORA LUTEA

| | Theca interna | Granulosa |
|---|---|---|
| Pig | + | o |
| Rabbit | o | + |
| Guinea pig | + | + |
| Dog | +, disappears after ovulation | trace |
| Rhesus monkey | + | + |
| Human | +, disappears after ovulation | o |

(Observations on dog, monkey, and man were limited in numbers.)

This seemingly erratic variation makes it difficult to theorize about the possible function of the alkaline phosphatase in the ovarian follicles. In the preliminary report of these studies (Corner, 1944) it was suggested that the enzyme, in tissues in which it is not associated with the storage of inorganic phosphorus, as it is in bone, may take part in reactions involving lipid metabolism. The same conjecture has been made by Emmel (1945), and by Hard (1946) in connection with his observations on alkaline phosphatase in the guinea pig's placenta. The latter author also suggests the possibility that the enzyme may participate in carbohydrate metabolism. Any possible relation of these functions to the production of ovarian hormones by the cell layers in question is even more nebulous.

In order to use the phosphatase content of the theca interna as a means of following the fate of its cells in the corpus luteum, it is necessary that the granulosa cells be free of phosphatase, and that the theca interna cells contain it and retain it during conversion of the follicle into the corpus luteum. These conditions prevail in the pig only, among the species examined in the present study.

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## Plate 1

All the figures of this plate represent ovaries of the pig. *gr.*, granulosa; *th. int.*, theca interna.

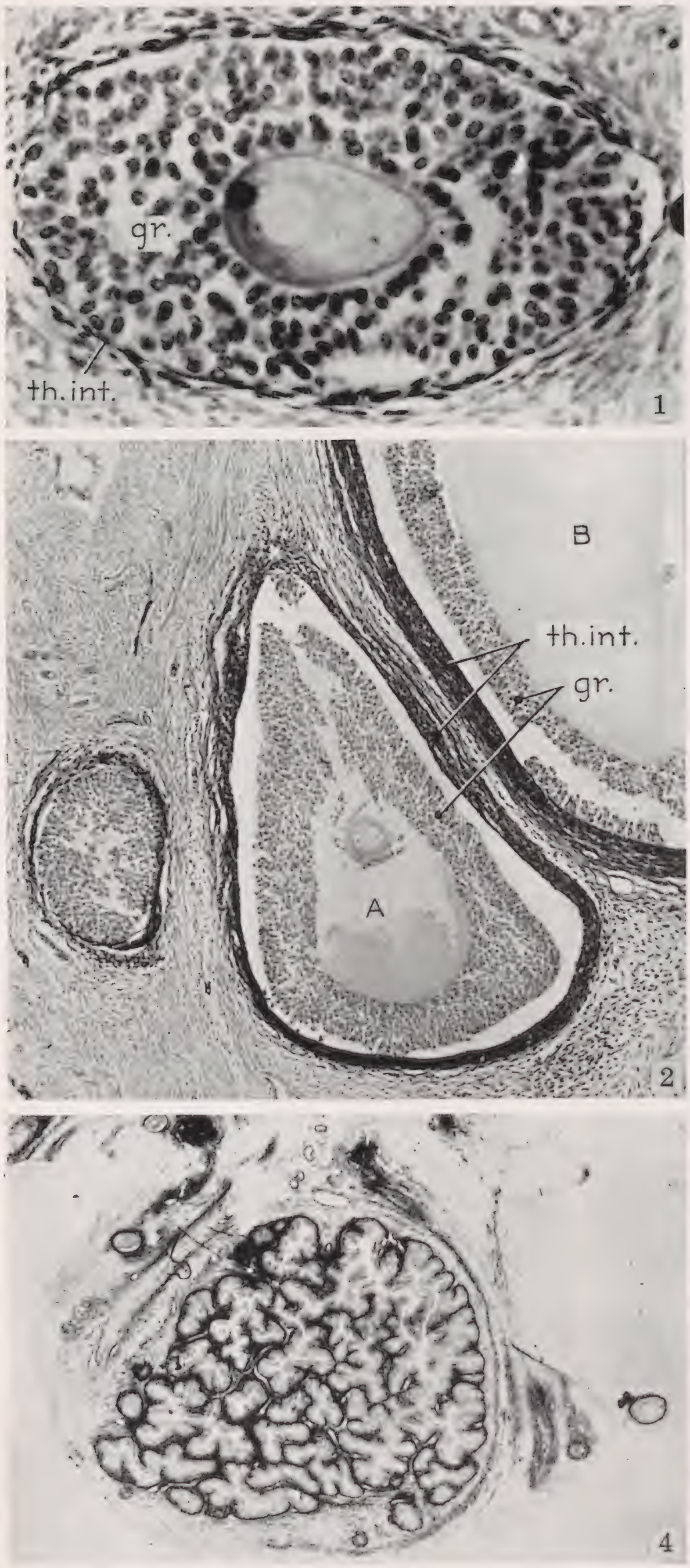
Fig. 1. Small follicle, ×375. Phosphatase in nuclei only of granulosa cells and theca interna cells.

Fig. 2. Phosphatase in cytoplasm of theca interna cells in 0.5-mm. follicle (*A*) and 5-mm. follicle (*B*). ×100.

Fig. 3. Mature follicle 10 mm. in diameter; part of wall ×300. Phosphatase in cytoplasm of theca interna cells.

Fig. 4. Corpus luteum about day 2 after ovulation, ×10. Phosphatase-laden theca interna makes a convoluted pattern throughout the young corpus luteum.

Fig. 5. Same specimen as figure 4, small area ×100.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B
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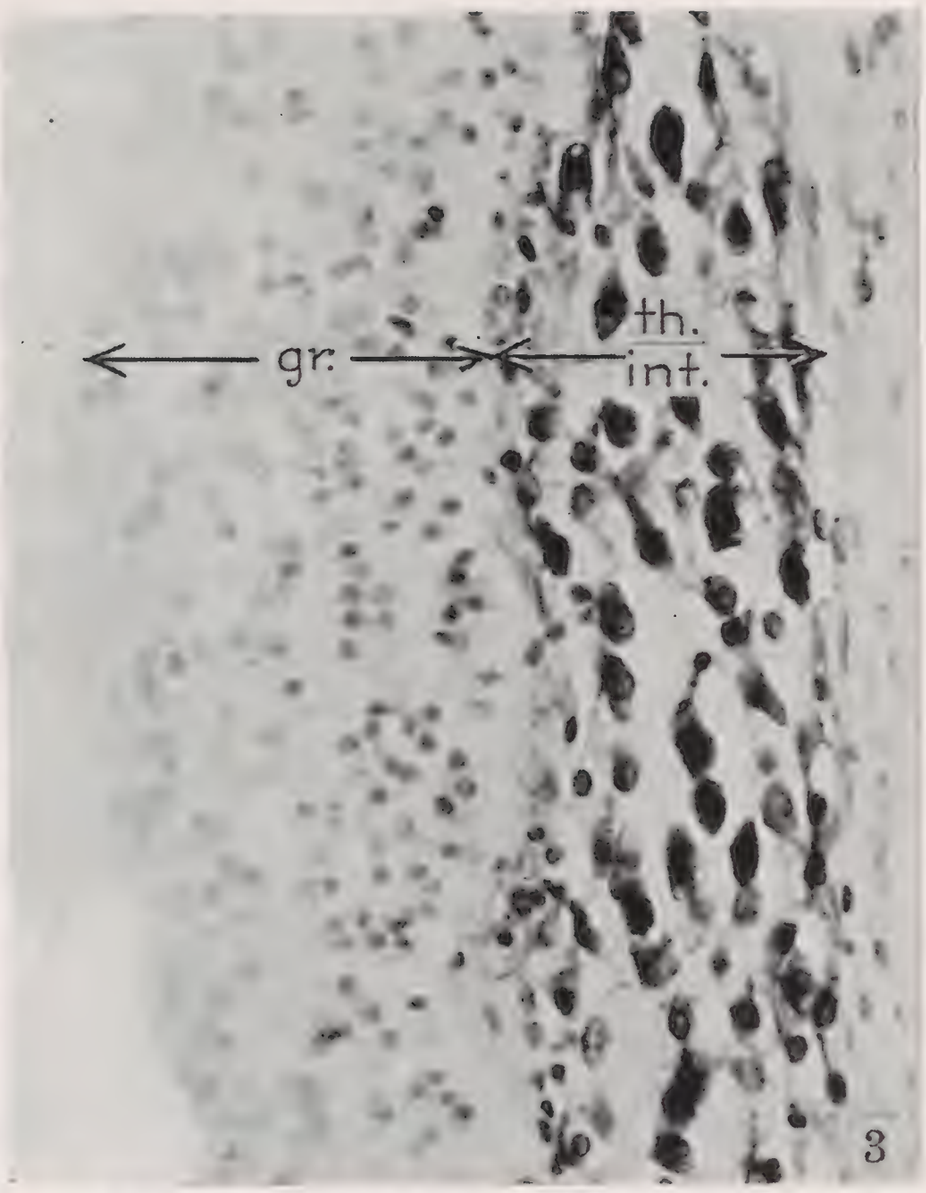
gr.
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th.int.
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5

## Plate 2

All the figures of this plate represent ovaries of the pig. *th. int.,* theca interna; *th. c.,* cells of thecal origin.

Fig. 6. Corpus luteum about day 3 after ovulation, ×100.

Fig. 7. Corpus luteum about day 4 after ovulation, ×250. Some of the theca interna cells are scattering through the granulosa.

Fig. 8. Corpus luteum, day 6 or 7 after ovulation, ×100. Blood vessels, with phosphatase-laden endothelium, and theca cells are wandering through the granulosa.

Fig. 9. Corpus luteum, day 7 or 8 after ovulation, ×100. The inner zone of the luteal layer (i.e. the half which lies nearer the former lumen of the follicle) is lighter in tone than the basal zone, because the blackened theca interna cells have not yet permeated the inner zone.

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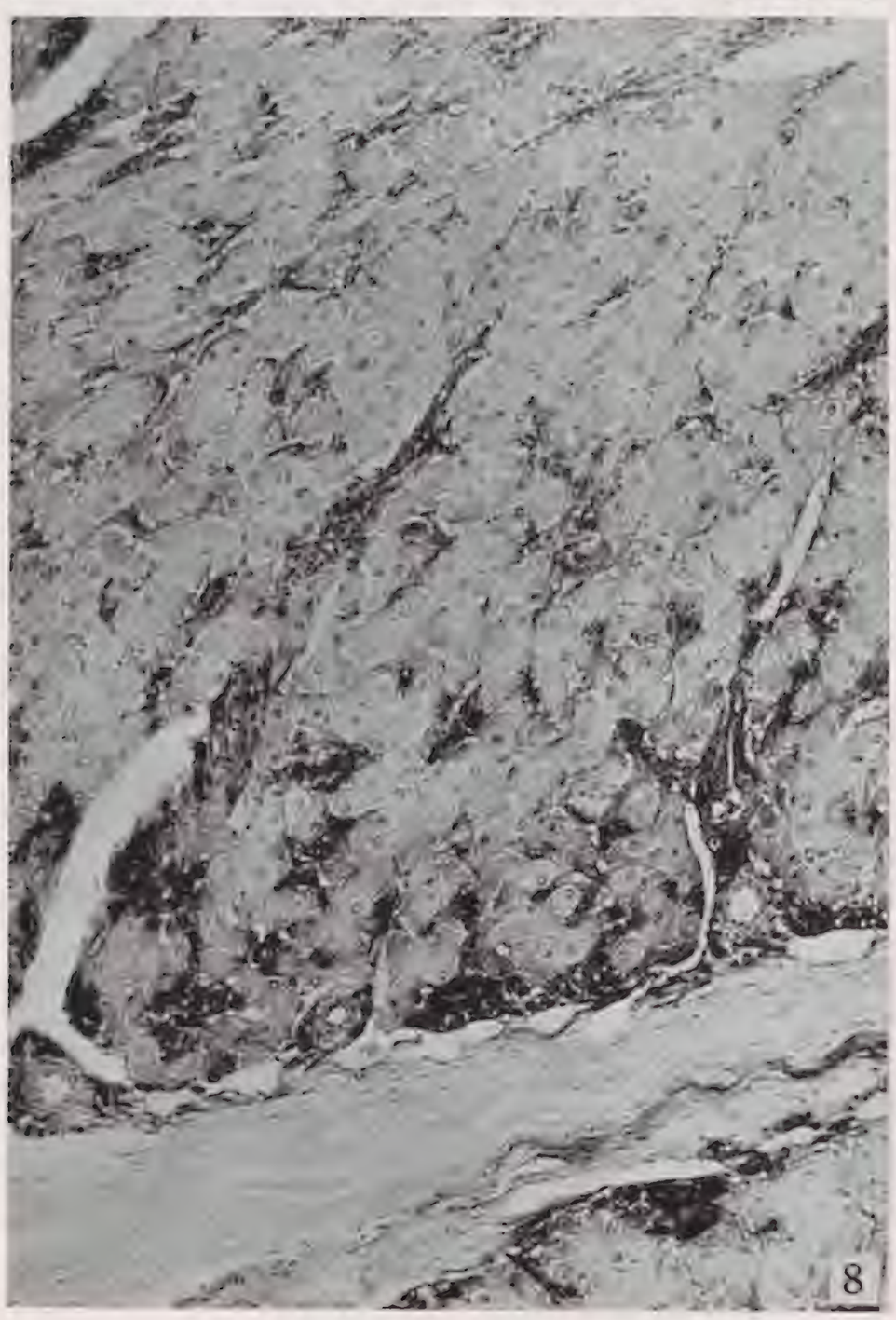

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## Plate 3

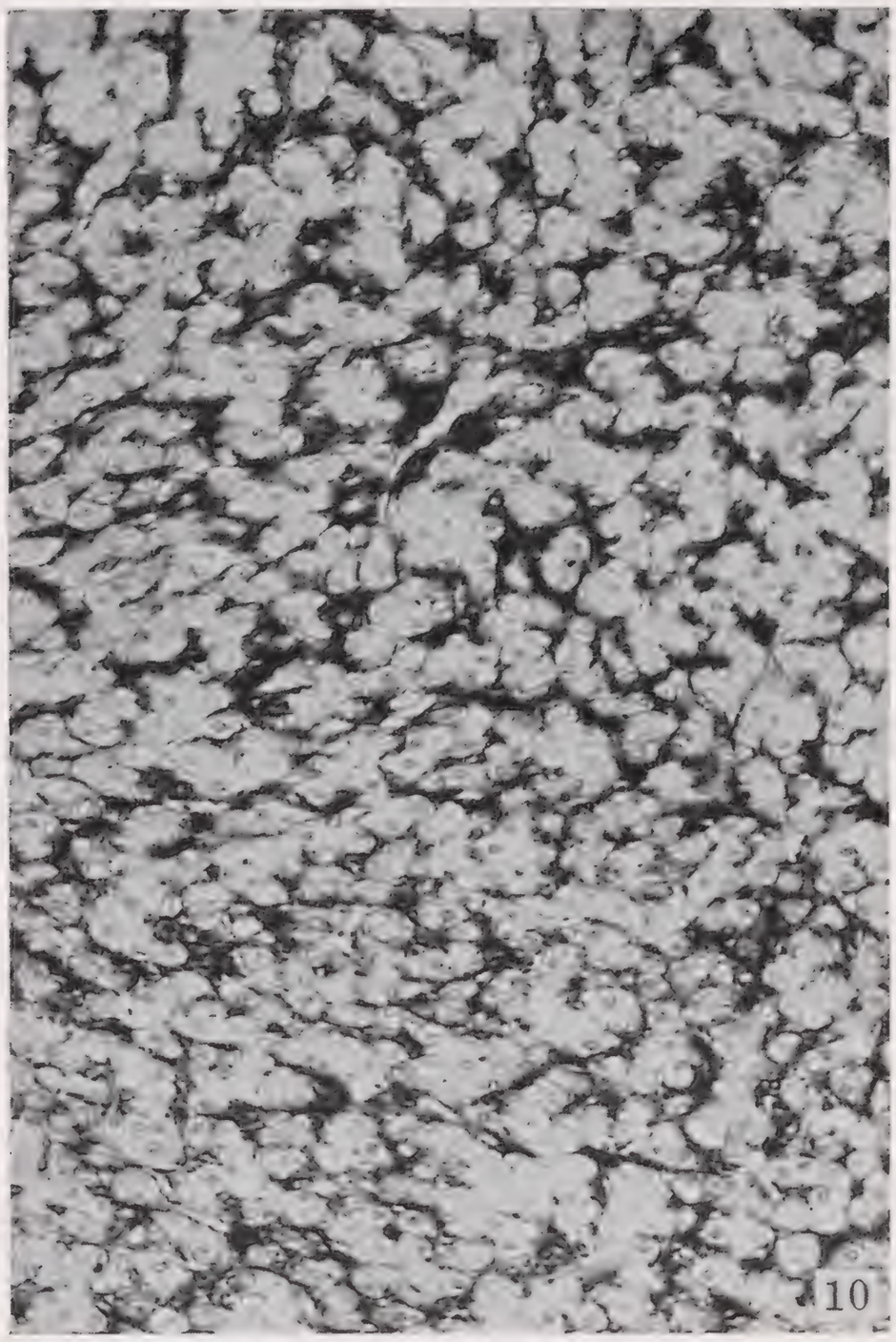
All the figures of this plate, except figure 13, represent ovaries of the pig. *gr.*, granulosa; *gr. c.*, cells of granulosal origin; *th. int.*, theca interna; *th. c.*, cells of thecal origin.

Fig. 10. Corpus luteum of pregnancy, 18 to 19 days, ×100. Phosphatase-laden theca interna cells, accompanied by phosphatase-laden endothelium of capillary blood vessels, are disseminated throughout the tissue.

Fig. 11. Same specimen as figure 10, ×600.

Fig. 12. Corpus luteum of pregnancy, about 7 weeks, ×600. Phosphatase is now appearing in some of the granulosa luteal cells.

Fig. 13. Wall of mature follicle, rabbit, ×725. Phosphatase in granulosa cells but not in theca interna cells.



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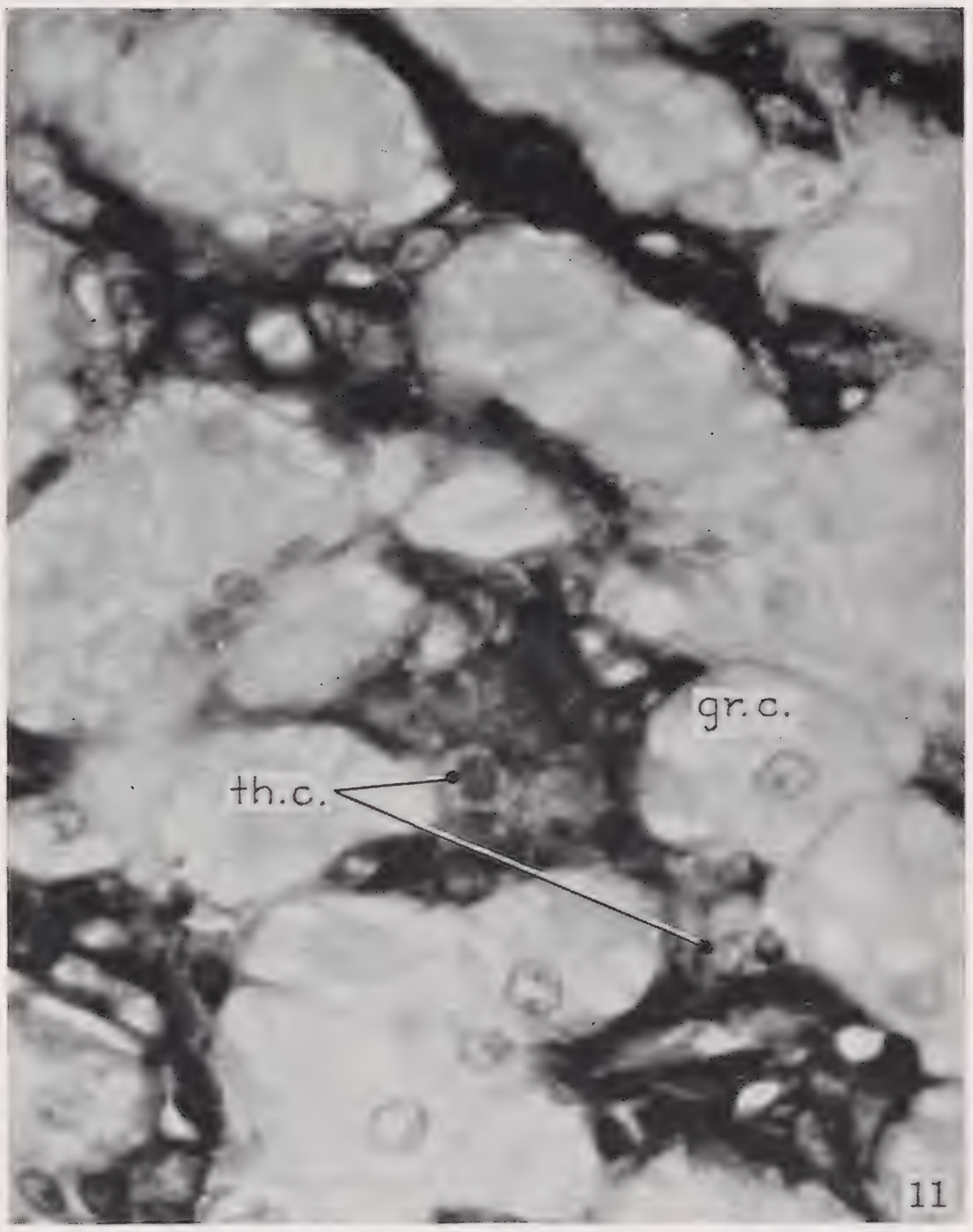


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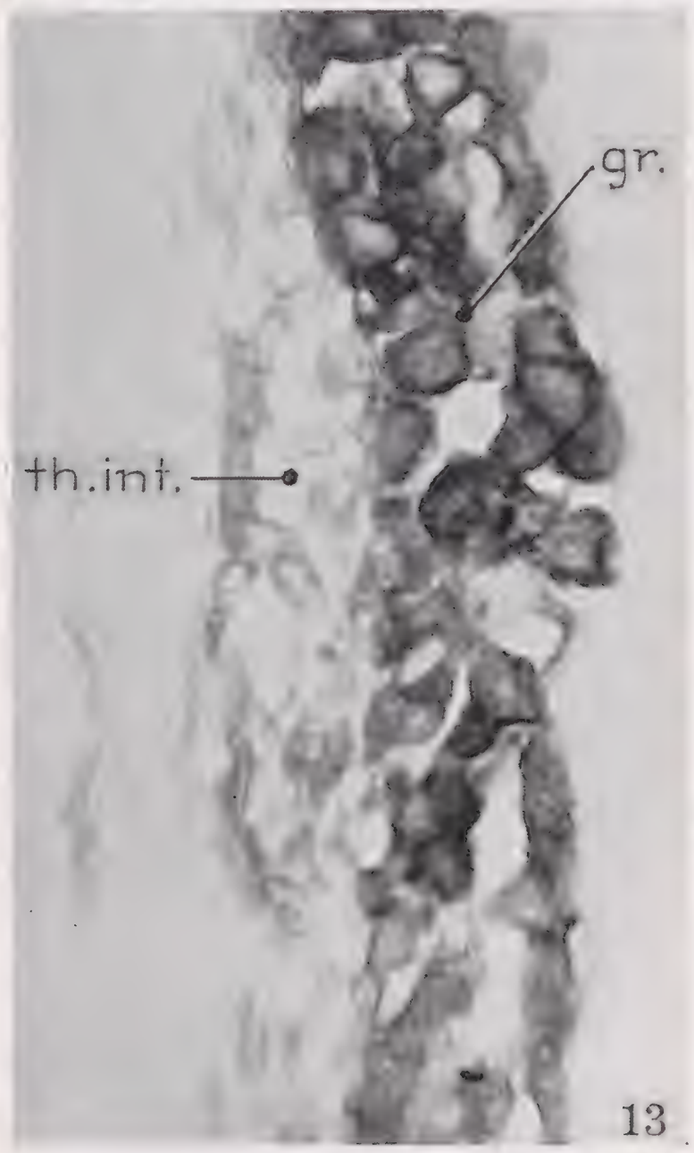


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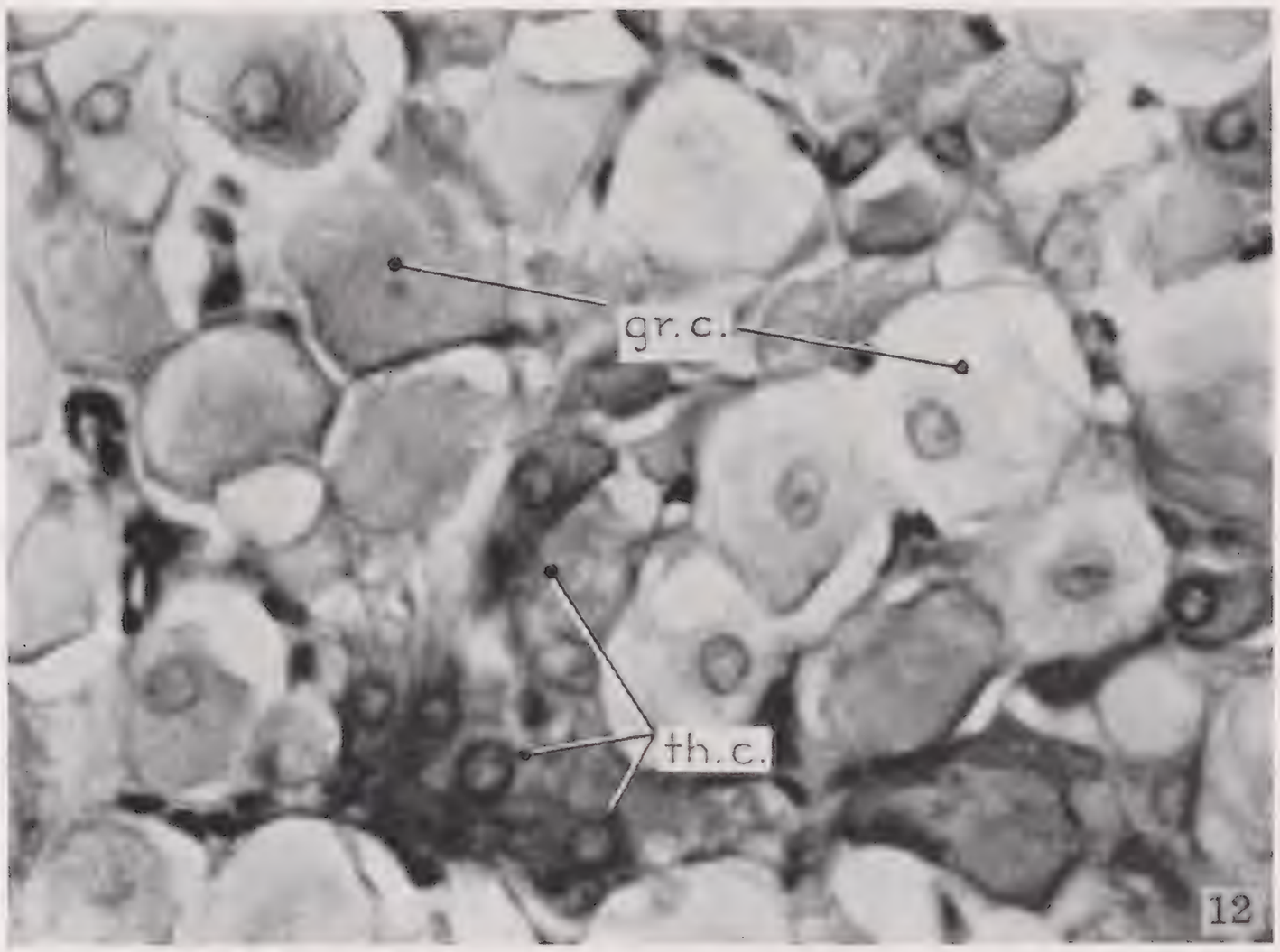


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## Plate 4

*gr.*, granulosa; *th. int.*, theca interna; *th. c.?*, cells probably of thecal origin.

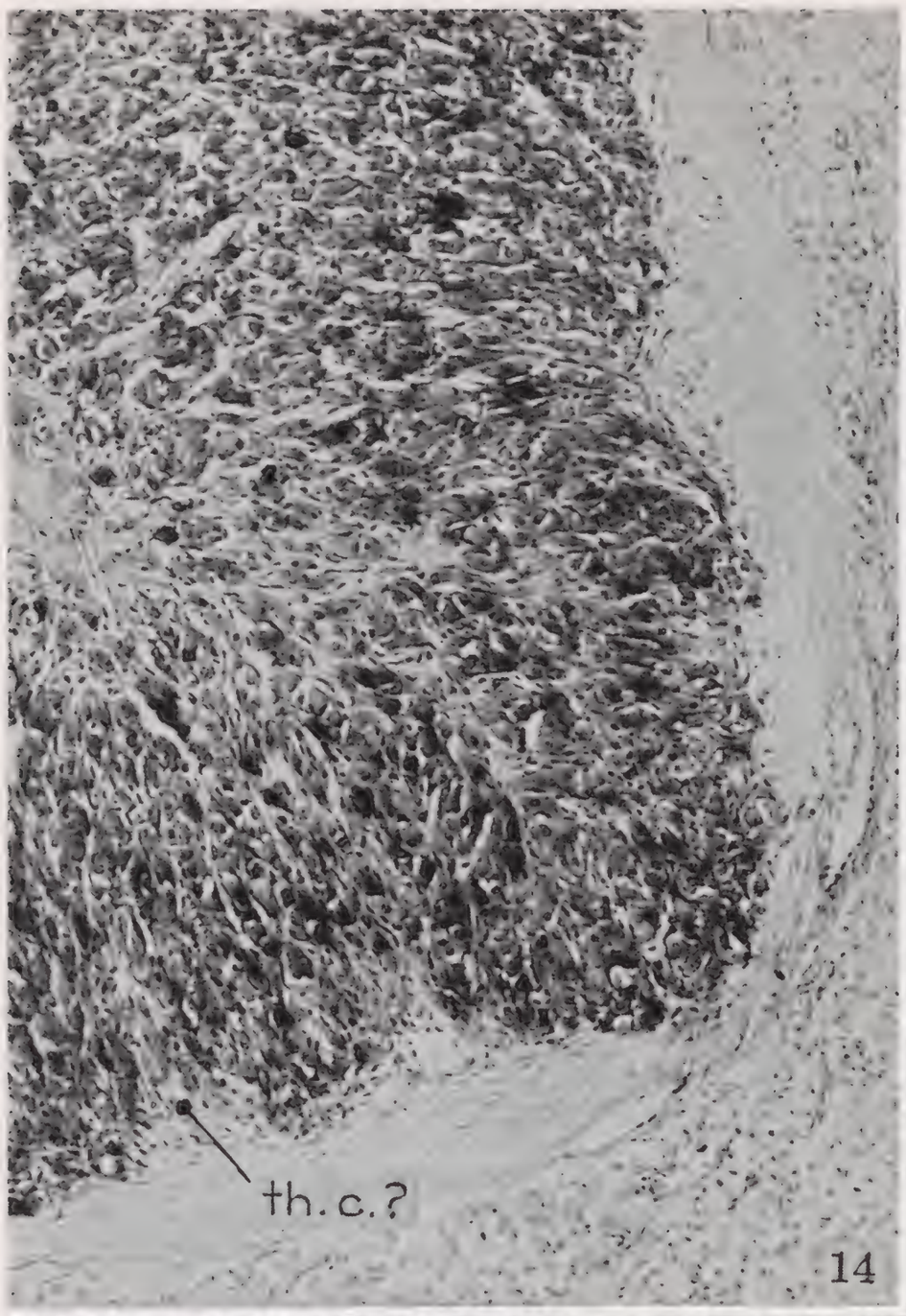
Fig. 14. Corpus luteum, rabbit, 3 days after ovulation, ×100.

Fig. 15. Maturing follicle, guinea pig, ×100. Phosphatase in both granulosa and theca interna.

Fig. 16. Corpus luteum, guinea pig, 3 days after ovulation, ×100.

Fig. 17. Mature follicle, dog, ×25.

Fig. 18. Same specimen as figure 17, ×200. Phosphatase in the theca interna, a trace in the granulosa.

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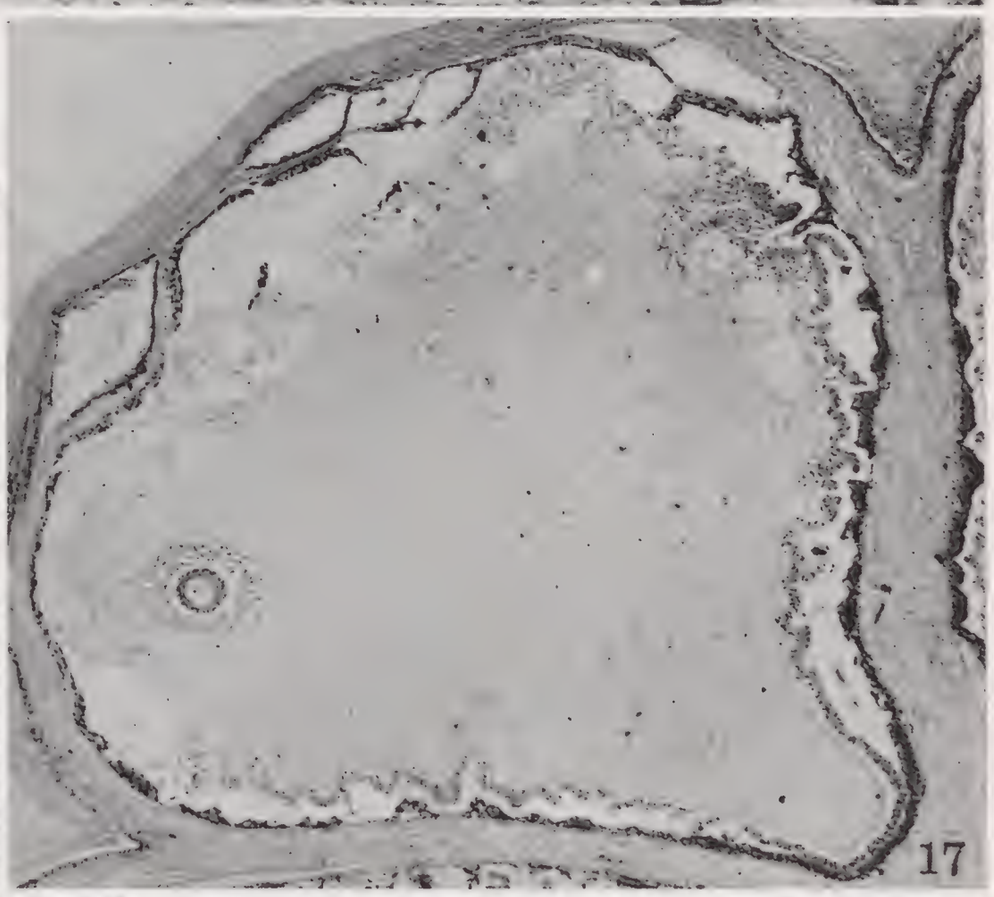

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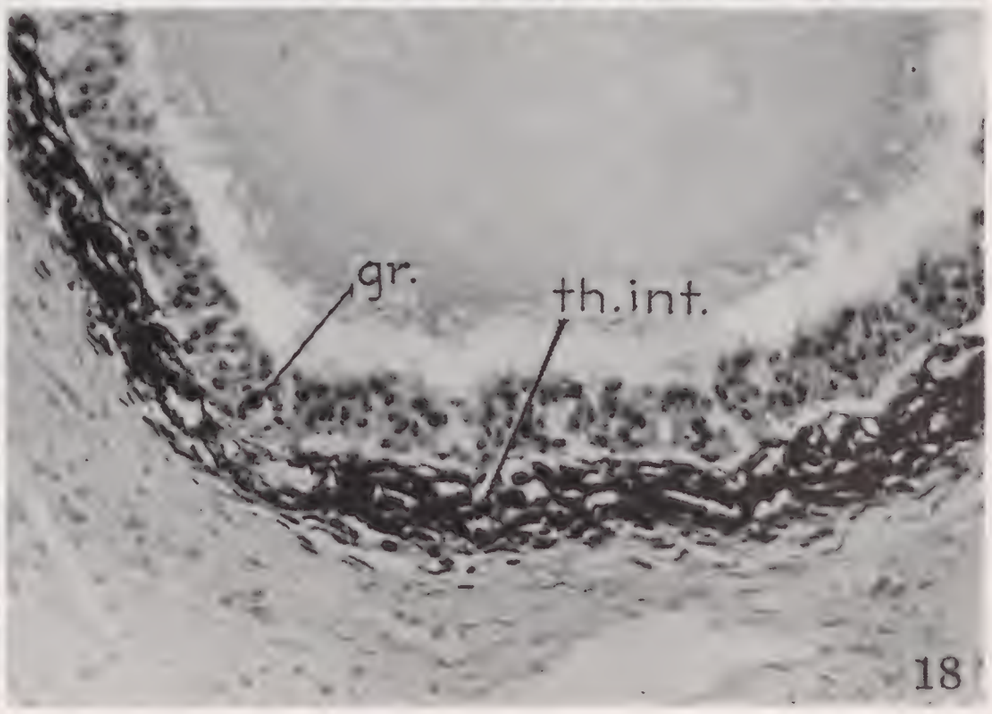


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## Plate 5

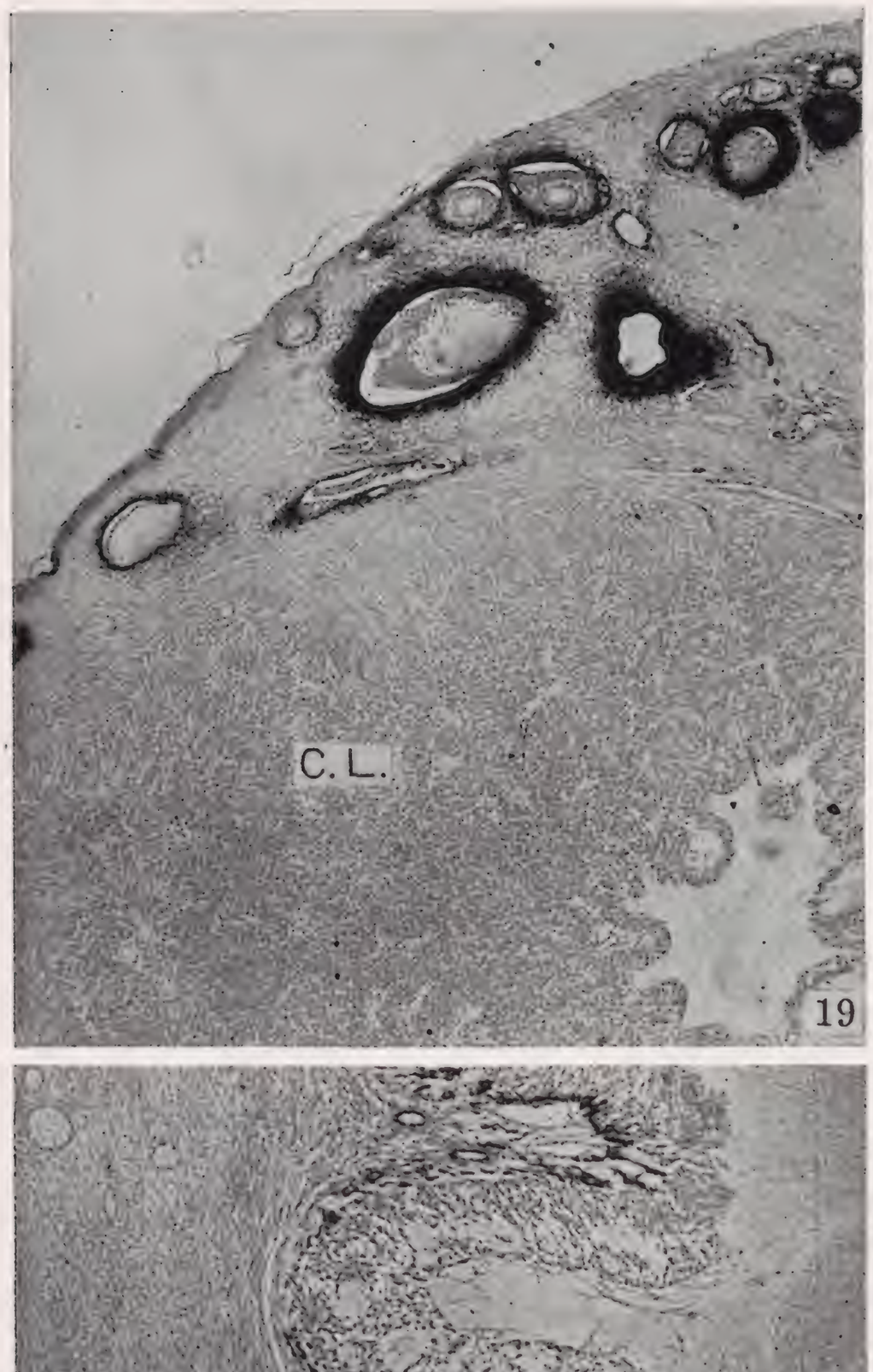
*C. L.*, corpus luteum; *gr.*, granulosa; *th. int.*, theca interna; *th. c.*, cells of thecal origin.

Fig. 19. Corpus luteum, dog, 4 or 5 days after ovulation, ×25. No phosphatase in corpus luteum except in endothelium of a few small blood vessels. Dense deposits of phosphatase in theca interna of small follicles, several of which are seen at the top of the picture.

Fig. 20. Ovary of rhesus monkey, 25 hours or less after ovulation, ×15. Phosphatase in both granulosa and theca interna of ruptured follicle.

Fig. 21. Human corpus luteum, about 1 day after ovulation, ×40. Phosphatase in theca interna.

Fig. 22. Human corpus luteum, about 5 days after ovulation, ×100. No phosphatase in cytoplasm of theca interna cells. Light deposits of phosphatase in a few granulosa cells near inner surface.

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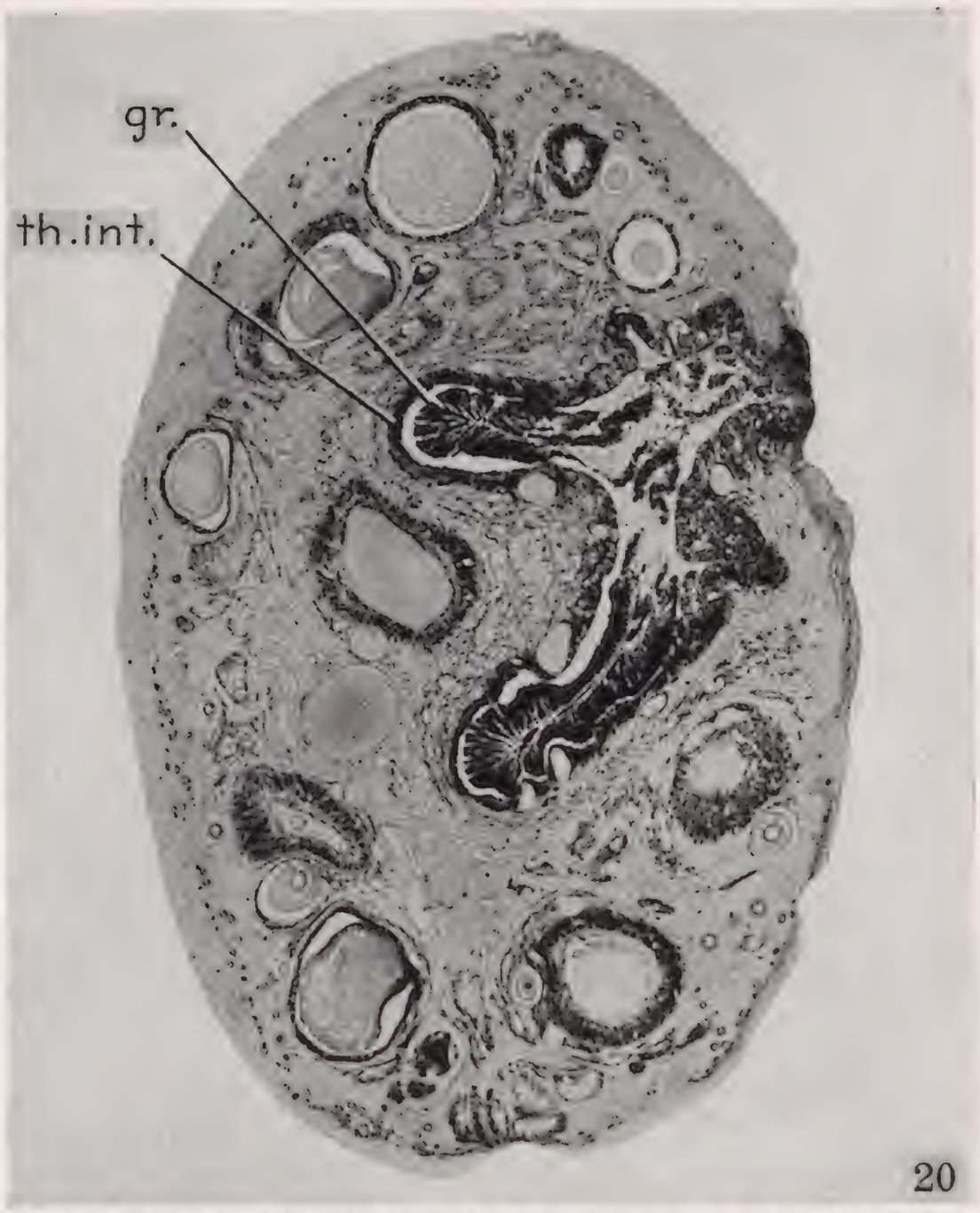


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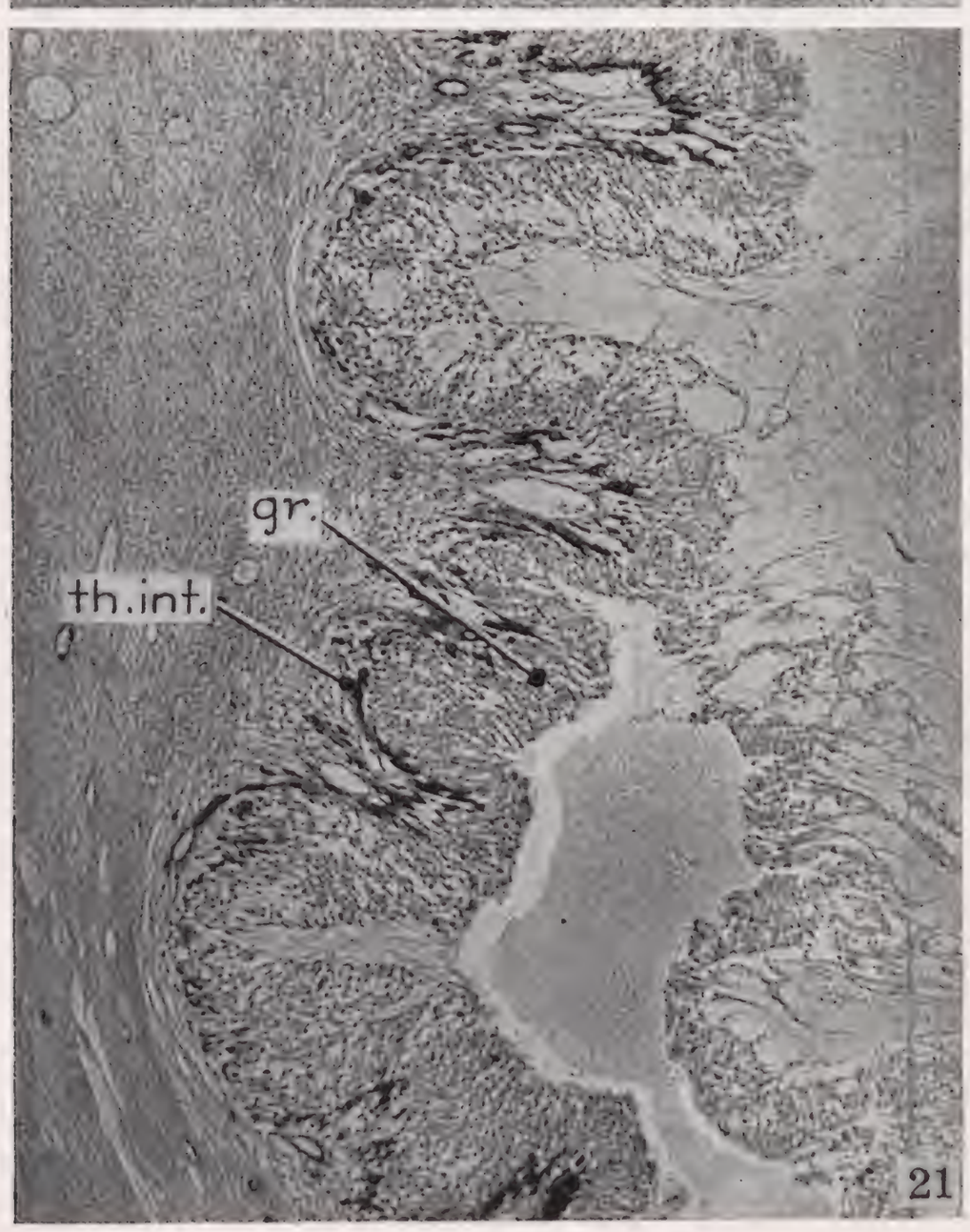


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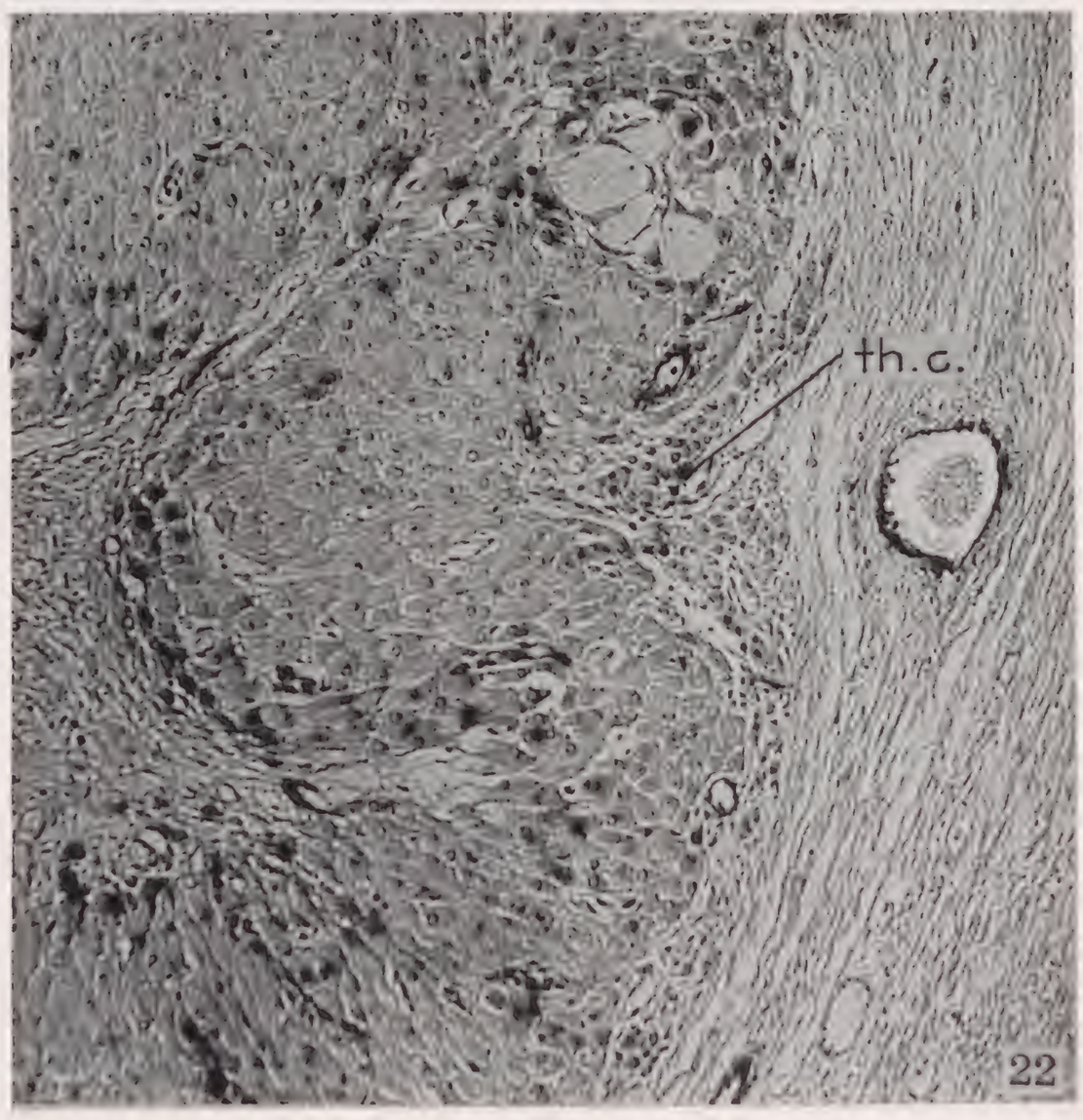


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CONTRIBUTIONS TO EMBRYOLOGY, NO. 208

# THE NORMAL AND EXPERIMENTAL DEVELOPMENT OF THE MAMMARY GLAND OF THE RHESUS MONKEY, WITH SOME PATHOLOGICAL CORRELATIONS

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With nineteen plates and three text figures

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# THE NORMAL AND EXPERIMENTAL DEVELOPMENT OF THE MAMMARY GLAND OF THE RHESUS MONKEY, WITH SOME PATHOLOGICAL CORRELATIONS

## ANATOMY AND DEVELOPMENT

### Embryo

In adult life, different species show marked differences with respect to number, size, shape, structure, and developmental physiology of the mammary glands. These differences extend back through early stages of development and serve to distinguish the various species even during fetal life. As one goes back toward earlier embryonic stages, however, a striking similarity appears in the organogenesis of the mammae. Numerous studies have been published, all in essential agreement, based on observations in the rabbit, guinea pig, mouse, rat, dog, cat, horse, cow, sheep, swine, goat, mole, fox, squirrel, porcupine, bat, opossum, kangaroo, and human.

The earliest primordium of the mammary apparatus consists of the milk streak, a zone of the integument characterized by an enlargement and elongation of the cells of the stratum germinativum. As the germinative cells proliferate, this paired epithelial structure, which runs longitudinally along the ventral surface of the embryo from the anterior to the posterior limb bud, develops into the Milchleiste or milk line. The localized elevations which appear along this line are the direct forerunners of the individual normal and supernumerary mammae of later life.

The mammary line was observed in the human for the first time in an embryo of 15 mm. crown-rump length (Kallius, 1897). Subsequent investigators have demonstrated this structure in earlier human embryos (Schmitt, 1898, crown-rump length 9.5 mm.; Brouha, 1905, C.-R.L. 9 mm.; Lustig, 1916, C.-R.L. 9.75 mm.). In the human, in contrast with the polymastic mammalian forms, Schmidt (1897) and Schmitt (1898) were able to demonstrate the milk line only in the pectoral region.

No observations have been recorded concerning the early development of the mammary apparatus of the rhesus monkey. A group of specimens in the Carnegie Embryological Collection was therefore examined, with the aim of determining the type and time of development of the mammary line of the macaque (table 1). All the embryos were studied in serial sections except C 499 and C 665. Stereoscopic photographs of the latter clearly revealed a definitive mammary primordium developing from the mammary line in the thoracic region, just caudad to the anterior limb bud (fig. 4, pl. 1). There was no gross evidence of a mammary line extending to the posterior limb bud. Histologic examination of C 479, on the other hand, showed a mammary line running along either side of the entire ventral surface of the embryo, from the anterior to the posterior limb bud, but best developed in the thoracic region. In cross section through this region the line was seen as a paired thickening of the epidermis projecting from the body wall, medial and dorsal to the limb buds (fig. 5, pl. 1). The adjacent epidermis was composed of two layers of cells, an internal stratum germinativum and an outer layer of periderm. The cells of the mammary line proliferate from the former. In stained sections the line presented a darker appearance than the surrounding tissues, because of its component mass of closely packed cells. Covering the milk line was the flattened layer of periderm (fig. 6, pl. 1).

TABLE 1

Monkey embryos examined for a mammary line

| Embryo | Crown-rump length (mm.) | Conception age (days) |
|---|---|---|
| C 501..... | 5.4 | 26½ |
| C 477..... | 7.4 | 29 |
| C 499..... | 8.0 | 31 |
| C 479..... | 11.3 | 34 |
| C 665..... | 11.9 | 35 |

The mammary line in the rhesus monkey was thus clearly demonstrated at a gestation age of 34 days (C.-R.L. 11.3 mm.), running along the entire ventral surface of the embryo. In the 11.9-mm. embryo the caudal part of the line had disappeared, at least in the gross. At earlier stages of development (C.-R.L. 8 mm., 7.4 mm., 5.4 mm.) this structure could not be identified. Specimens were not available for study of mammogenesis beyond the 35th day of development.

## Newborn

The mammary glands of the human at birth are usually larger than those encountered in infancy and early childhood. In microscopic section the duct epithelium is found to be hyperplastic, containing numerous mitotic figures, and secretory activity is often present in the alveolar cells (Keiffer, 1902). These manifestations of mammary activity form but a part of the general pattern resulting from intense stimulation of the entire genital system of the fetus during late pregnancy, including edema of the labia or scrotum and enlargement of the uterus (Halban, 1904). During the early neonatal period uterine bleeding (menstruatio neonatorum) and mammary secretion (witches' milk) are frequently observed, the latter having been noted in some children for as long as 8 months (Hoeland, 1927). These phenomena are usually attributed to the hormonal stimulation of pregnancy, and authors have referred to them as manifestations of a fetal response to maternal estrogens, since these hormones appear in equal concentrations in maternal and fetal blood. The presumption is very strong that the placenta is the source of the high estrogen titer of pregnancy. Since the placenta is truly a fetal organ, however, the estrogens of pregnancy might be referred to more properly as fetal than as maternal hormones. The latter designation is probably incorrect.

In common with all other primates, the rhesus monkey normally has but a single pair of mammary glands, situated in the pectoral region. (The lemurs have also a pair of inguinal or "anchoring" nipples, which are used for the attachment of the young, but no mammary parenchyma is associated with them.) Our material has included the mammary glands of 7 newborn monkeys. At birth the mammae formed inconspicuous structures, their only external manifestation being a small areola. The nipple was practically flush with the skin, and no mammary tissue was palpable. By careful dissection under the binocular microscope the gland was found lying in a thin plane of connective tissue adherent to the skin, and measured 2 mm. or less in diameter. The parenchyma consisted of but a few short, poorly branched ducts, with no semblance of lobular structure. A typical specimen is shown in figure 7, plate 1. When viewed in cross section the ducts were found to be composed of hyperplastic epithelium, four to six cells thick. The cells and nuclei were large, and mitotic figures were often seen. The lumina of the ducts were empty and there was no evidence of secretory activity in the cells (fig. 8, pl. 1). The gland consisted entirely of ducts at birth; no alveoli had formed as yet. The stroma was of an undifferentiated type and lacked the extramedullary hemopoiesis which has been described in the breast of the human newborn (Gruber, 1921).

## Adolescent

From such a simple, undifferentiated duct system the complex mammary tree of the adult, with its constituent lobules of alveoli, gradually unfolds. The hyperplastic epithelium seen in the duct linings of the newborn involutes during the neonatal period; the ducts of older infants were lined with only one or two layers of cells. With the general somatic growth of the animal, branching and extension of the mammary ducts occurred, causing an increase in the area of the glands. Measurements of area have served as the sole indicator of the growth of the mammae. This has seemed entirely adequate, however, for during its prepregnancy development, increase in thickness of the mammary gland is neither striking nor easily measurable.

The right and left glands increased in area at about the same rate, so that there was never any significant difference in size between them. In contrast with the constant rate of growth of the kidneys from birth, Aberle (1934*b*) found the highest growth rate for the ovaries and mammary glands at puberty. The mammae of 3 2500-gm. females were found to average 4 sq. cm. in area. Increase in size occurred up to a body weight of about 5000 gm., at which stage of development the average gland area of 7 animals was 100 sq. cm. Folley, Guthkelch, and Zuckerman (1939), from the measurements of 26 preparations from nonpregnant rhesus monkeys weighing between 2200 and 7100 gm., have even thought it possible to reduce the growth of the mammary gland to the simple formula $A=0.0000138W^{1.79}$, over the body-weight range 3400 to 6060 gm., $A$ representing the

area of the gland and *W* the body weight of the animal. These latter authors have also denied the occurrence of alveoli in the mammary glands of monkeys whose ovaries show no signs of either existing or previous luteinization. Their observations, however, were probably based upon too small a series of animals; we have frequently encountered lobules of alveoli in prepubertal monkeys the ovaries of which showed no trace of ovulation. As will be described in detail later, marked variation in this respect has been observed. On the other hand, even in parous monkeys complete lobular atrophy of the mammary glands may be anticipated in the absence of sustained ovarian stimulation.

## Adult

The breast of the rhesus monkey normally has a single nipple containing two to ten excretory ducts. In the adult the nipples average about 10 mm. in length and are located at about the level of the sixth costochondral junction, their distance above the pubic symphysis averaging about 82 per cent of the anterior trunk height (Schultz, 1924). Wide variations may occur, however, both among individual animals and between the two nipples of the same animal, depending upon the mechanical stimuli to which they have been subjected, especially during lactation. Each nipple is surrounded by an areola of variable size. Both structures take on a deep red coloration in response to the intense hormonal stimulation of pregnancy and the mechanical stimulation of suckling.

As in many other mammals such as the rabbit, mouse, and rat, but not as in the human, the mammary gland proper lies in a flat sheet of connective tissue beneath and in close apposition to the skin. In fully mature animals the gland may extend above and laterally into the axilla and below to the costal margin. A variable number of ducts radiate from the base of the nipple; each branches repeatedly, forming a complex duct tree from which the lobules of alveoli are ultimately derived (fig. 9, pl. 2). Surrounding the lobules is a loose connective-tissue stroma through which course the blood vessels. Each lobule is supplied with one or more arteries which break up into a dense system of capillaries going to the individual alveoli. The ducts are also surrounded by a delicate network of fine vessels. An excellent idea of the high degree of vascularity of the gland may be obtained from figure 10, plate 2, which shows a region of a gross mount following intravascular injection in vivo with India ink.

In nonpregnant animals the limits of the mammary glands can neither be seen nor determined by external palpation. During the latter part of pregnancy, however, the glands gradually increase in thickness, becoming visibly and palpably enlarged when lactation sets in. When lactation is at its height the milk-filled mammae assume greatly increased proportions and may hang in large folds below the costal margins (fig. 11, pl. 2).

## Individual Variation

No organ undergoes more profound changes in the course of its normal development than does the mammary gland. It ranges in its physiologic responses to hormonal stimuli from the sparsely branched, naked duct system of infancy to the dense lobules of secreting alveoli characterizing late pregnancy and lactation. The nature and tempo of the various phases of mammary development and involution have naturally attracted wide attention, as is evidenced by an extensive literature. Studies on various mammals are, for the most part, in good agreement, for it has been possible to obtain mammary specimens from them under well controlled conditions. Despite the greater interest in the human breast and the numerous studies which have been made upon it, however, uncertainty still exists concerning the details of many of its developmental changes. Divergent views are still held, for example, concerning the existence and nature of cyclic changes in the human mammary gland in relation to menstruation (Speert, 1941).

There are several reasons for the difficulties encountered in studies of the human mamma. Because of its large size and conical shape it is difficult to study the gland in its entirety, as is possible with whole mounts of the glands of many laboratory animals. Thick sections of the whole breast or microscopic sections of selected regions must be relied upon (Ingleby and Holly, 1939). Study of the latter must

be undertaken, however, with the full realization that wide variations may be encountered in different regions of the same breast. An equally serious difficulty is inherent in the nature of most of the material which has been studied. Many specimens, for example, have been obtained from patients at operation for mammary disease; others have been obtained at autopsy following debilitating illnesses. Few specimens could be judged completely normal. The greatest difficulty in the study of human mammary glands, however, probably lies in the tremendous range of variation which may exist among normal individuals. This has vitiated, in many instances, conclusions based upon the comparison of glands from different women.

As a result of his autopsies, Engel (1941) called attention to the great variation in structure and amount of glandular tissue present in the breasts of 26 women who died intrapartum or in the first few days of the puerperium. Yet too little recognition has been accorded this important factor of individual variability in the female mammary gland. The most widely quoted papers which purport to demonstrate a cycle in the human breast and its development during pregnancy have been based on specimens obtained from numerous women, each representing a different stage. Likewise, the demonstrated effects of various hormones upon the mammary gland may be questioned when the control and test specimens have been obtained from different individuals.

The present studies were also begun without full appreciation of the importance of this factor of individual variability. It was expected that numerous questions relating to physiologic and experimental processes in the mammary gland could be answered from the study and correlation of the large amount of material on hand. Since all stages of the menstrual cycle, pregnancy, and involution were represented, it was anticipated that the reflection of these processes upon the mammary gland could be studied by examination of the appropriate specimens. The material consisted almost exclusively of glands which had been removed at autopsy, however, and in only a few instances were previous biopsy specimens available from the same animal. After the laborious preparation of a large number of gross mounts as well as histologic sections, it was disappointing to find that no clear sequence of changes could be demonstrated when the specimens were arranged according to the stage of the menstrual cycle, pregnancy, or the puerperium. Moreover, wide differences existed among the glands of both normal and castrated animals which had received similar treatment with various endocrine preparations.

In several instances many specimens were available which represented identical physiologic states, such as the same day of the menstrual cycle, pregnancy, or the puerperium, in different animals. When the mounts and sections of these glands were compared it was immediately apparent that the most marked individual variations existed. Only after a long period of castration, advanced pregnancy, or prolonged estrogenic treatment could a specific mammary pattern be anticipated with reasonable assurance. A significant range of individual variation was encountered under all other circumstances.

The large majority of the mammary glands were from mature females. Examination of a smaller number of mammae obtained from younger animals also revealed a striking range of individual variation. Figures 12 and 13, plate 2, for example, show the mammary glands of two preadolescent monkeys of approximately the same size, weighing 3150 gm. and 3220 gm. respectively. The ovaries of both animals were juvenile, showing no trace of previous ovulation. The great difference in size and development of the mammary glands is apparent in the figures. Equally striking were the extremes which were encountered in a series of mammary glands obtained from 24 adult male monkeys (see section on the male gland). In an earlier study of mammary development in the rhesus monkey, Aberle (1934*b*) also mentioned the individual variation in the amount of parenchymal tissue found in a small series of prepubertal females.

The largest group of specimens representing the same physiologic state in the female were those obtained 11 days after ovulation. Eighteen such specimens were studied, most of which were obtained for biopsy at the time of laparotomy for the recovery of the fertilized ovum. In each instance the diagnosis of ovulation was made preoperatively by Dr. Hartman, by means of bimanual palpation, and was confirmed at operation.

Comparison of these specimens revealed all gradations of development, from thin glands with small, sparsely distributed lobules of collapsed alveoli and much interlobular stroma to thick glands composed

of hypertrophic lobules of patent alveoli which almost completely replaced the interlobular stroma. Figures 14 and 15, plate 3, are mounts prepared from biopsy specimens which clearly show these two extremes. Both were taken from healthy mature female monkeys under physiologic conditions which were apparently identical. The impossibility of demonstrating different stages of a physiologic process by comparing specimens obtained from two such animals is evident. The degree of individual mammary variation between these two animals is much greater than any change which normally occurs in the gland during the course of a menstrual cycle. This obstacle has been circumvented in the present studies by means of multiple serial biopsies in the same animal, at various phases of mammary development or before and after experimental procedures. Biopsies were performed upon alternate breasts. In this manner, as many as 10 mammary specimens have been obtained from a single animal.

## Supernumerary Mammae

Although two is the number of mammary glands characteristic of primates, several instances of supernumerary nipples have been reported in various species besides the human. These are summarized in table 2.

All supernumerary nipples in primates, including those in the present series and the ones reported in the literature, have been below the normally situated nipples. In the human the great majority of supernumerary mammae likewise occur below the normal breasts (98.7 per cent, Kajava et al., 1921), although the reverse seems to hold for the Japanese (88 per cent of the supernumeraries are above the normal breasts, Iwai, 1907). The supernumerary nipples tabulated here have all conformed to a position along the approximate course of the embryonic milk line, extending from axilla to groin. This is also the commonest site for accessory mammae in the human (axillary, thoracic, abdominal, inguinal, and vulvar). In these positions, supernumerary mammae clearly

TABLE 2

Supernumerary nipples reported in primates

| Species | Author | Year | Sex | Side |
|---|---|---|---|---|
| Cercopithecus schmidti | Beddard | 1901 | Female | Bilateral |
| Cercopithecus patas | Sutton | 1889 | Male | Left |
| Callithrix | Schickele | 1899 | ..... | Left |
| Cebus capucinus (2 cases) | Schickele | 1899 | ..... | Bilateral |
| Cebus fatuellus | Schickele | 1899 | ..... | Left |
| Cebus hypoleucus | Schickele | 1899 | ..... | Bilateral |
| Papio porcarius (3 cases) | Zuckerman | 1935 | Female | 1 bilateral |
| Pithecus satyrus | Owen | 1886 | ..... | Left |
| Macacus sinecus | Sutton | 1889 | Female | Left |
| Macacus cynomolgus | Schickele | 1899 | ..... | Left |
| Macacus nemestrinus | Zuckerman | 1935 | Female | Right |
| Macaca mulatta | Hartman | 1927 | Female | Left |
| Macaca mulatta | Zuckerman | 1935 | Male | |
| Hylobates lar | Schultz and Coolidge | Personal communication | Female | Left |
| Symphalangus | Coolidge | Personal communication | Female | Right |
| Chimpanzee (2 cases) | Elder | 1936 | Female | Bilateral |
| | | | Male | Bilateral |
| Chimpanzee (2 cases) | Coolidge | 1933 | Female | Bilateral |
| | | Personal communication | Female | Right |

In a review of the mammary glands and protocols of approximately one thousand monkeys which have been recorded in the Carnegie Embryological Laboratory, thirteen cases of hyperthelia were encountered, in addition to the case previously reported by Hartman (1927). This gives an incidence for the rhesus monkey of 1.4 per cent, a figure in general agreement with the best estimates of this condition in the human. The number and arrangement of the supernumerary nipples have been described briefly in an earlier report (Speert, 1942*a*) and are presented in detail in table 3.

represent an abnormal persistence of embryonic rudiments. Yet occasional cases have been reported of aberrant breasts on the face, ear, neck, arm, thigh, and buttock, for which no satisfactory embryologic explanation has been found.

The supernumerary nipples in the present series were, in most instances, distinctly smaller than the normal ones. A typical example is shown in figure 16, plate 3. All gradations in size were encountered, however, the specimens ranging from a mere accessory areola (no. 593) to a nipple equal in size to the normal ones (no. 708).

Histologically, the supernumerary nipples were indistinguishable from the normal ones, except in a few instances where the surface epithelium seemed to be thinner in the former. In six cases mammary parenchyma was associated with the accessory nipple. This consisted usually of only a few short ducts, entirely distinct from the normal gland (fig. 17, pl. 3).

The hereditary nature and familial occurrence of supernumerary mammae has been recognized in sheep (Castle, 1924), swine (Wentworth, 1916), chimpanzee (Elder, 1936), and man (review by Speert, 1942*a*). A considerable body of suggestive evidence has arisen associating this condition also with an increased incidence of twinning. Monkeys nos. 272 and 273 in the present series arrived together in the laboratory from an animal dealer in New York City. Each had bilateral supernumerary nipples, and they appeared so similar in all other respects that they could not be distinguished. In the absence of accurate data it is impossible to state that they were twins, but the presumption is strong that they at least had a common parentage.

Supernumerary mammae appear to be biologically similar to the normally situated ones in their response to hormonal stimuli. In the human, for example, the presence of axillary breasts is often discovered for the first time toward the end of pregnancy, and if the gland has a nipple, lactation ensues following parturition. Zuckerman observed an increase in the prominence and deepening in the color of the accessory nipple in his male rhesus monkey following a series of injections with estrin. When the accessory nipple of monkey no. L1 in the present series was painted daily with an alcoholic solution of estrone (0.05 mg. per cc.) for 44 days, a marked increase in size and color occurred, the response being comparable to that which we have observed following similar treatment of normal nipples. The large accessory nipple of no. 708 failed to respond to the local application of estrogenic hormone, perhaps because of its having already realized its maximal possibilities for growth. Examination of the underlying gland, however, revealed the presence of extensive arborization of the ducts and many end buds, suggesting that parenchymal growth had probably occurred in response to the locally applied estrogen. Such treatment has previously been found effective in causing mammary growth in prepubertal male monkeys (Speert, 1940*b*). The gland's responsiveness to estrogen is unaffected by its location.

TABLE 3

MONKEYS WITH SUPERNUMERARY NIPPLES (PRESENT SERIES)

| Monkey | Sex | Side | Gland | Remarks |
|---|---|---|---|---|
| L1 | Male | Right | Present | Painted with estrone |
| G21 | Female | Left | Absent | |
| 118 | Female | Right | Absent | |
| 224 | Female | Right | Present | |
| 272 | Female | Bilateral | Right: present<br>Left: absent | Twins (?) |
| 273 | Female | Bilateral | Absent | Twins (?) |
| 313 | Female | Left | Present | |
| 418 | Female | Left | Absent | |
| 593 | Female | Left | Absent | Accessory areola |
| 690 | Female | Right | Present | |
| 695 | Female | Left | Absent | |
| 708 | Female | Right | Present | Painted with estrone |

## THE MALE GLAND

The male members of all mammalian species have mammary glands, which are numerically equal to although usually smaller than those of the female. The mammae are thus a class character rather than a sexual character, although in some animals (e.g., the mouse) nipples may be lacking in the male.

The structure of the male mammary gland in the human has been the subject of relatively little interest, and there is little general agreement at present as to what constitutes its normal anatomy. Langer (1852) studied injected and macerated specimens and reported the presence of knob-shaped endings on the small ducts but never any alveoli. At about the same time Luschka (1852), contrariwise, described alveoli in the male breast, although they were sparsely distributed and without lobular arrangement. Subsequent reports have failed to clarify this point. Kölliker (1880), for example, found the breasts of males between the ages of 20 and 30 years to be relatively well developed in size, with extensive branching of the ducts, but neither lobulated nor containing alveoli. Geschickter (1943) has not observed lobules in the normal male breast. Andrews and Kampmeier (1927), on the contrary, have stated that a complex system of ducts and alveoli exists in every male breast.

Under conditions of abnormal hormonal stimulation, such as occur in association with testicular tumors, the male mammary gland may attain extensive development and give rise to the breast enlargements which are designated clinically as gynecomastia. Haenel (1928) has even reported a case of bilateral lactation in a male which persisted for 20 years in association with a hypophyseal tumor. Such cases are clearly within the realm of pathology. Yet it has not been easy to make a sharp differentiation between normal and abnormal mammary glands in the male solely on the basis of the extent of their development, for great variations occur in this respect. In 1845 Sir Astley Cooper wrote of the male gland: "It varies extremely in its magnitude, in some persons being only the size of a large pea, in others an inch in diameter, and I have seen it two inches or rather more, and then it reaches even beyond the margins of the areola." Langer (1852) stated that he had never met with such numerous variations as in the extent of the duct development in the male breast. Direct measurements are in agreement with such statements, for Henle (cited by Kölliker, 1880) has found the adult male breast to range in diameter from 6.5 to 46 mm., and Gruber (cited by Kölliker, 1880) has observed variations between 1 and 137 gm. among 80 male breasts which he weighed.

In an effort to determine the extent of normal mammary variation in the adult male rhesus monkey, the glands of 24 fully mature animals were studied. In the monkey, as in the human, extreme variations in size and development were noted.

Folley, Guthkelch, and Zuckerman (1939) have stated that the mammary gland of the male macaque consists of a relatively uncomplicated duct system which does not extend far beyond the base of the nipple. Most of their 5 animals were not fully mature, and the clumps of alveoli which appeared in the breast of one animal were regarded as a manifestation of gynecomastia. We have found, on the other hand, in agreement with Gardner and Van Wagenen (1938), wide variations in the area of the normal male breast. The diameters of the glands ranged from a minimum of 3 mm. to a maximum of 31 mm. Our material has revealed, in addition, extreme variability in regard to the number of ducts, their caliber, the extent of branching, and the presence of end buds and lobules of alveoli.

Representative glands are shown in figures 18 to 27, plate 4. These pictures illustrate better than can any verbal description the range of variation in the male mamma. It is immediately apparent that no single pattern can be regarded as characteristic of the adult male monkey. All gradations were observed between the tiniest gland, with bare unbranched ducts which did not extend beyond the nipple base, and the largest gland, with extensive arborization of the ducts and definite lobules of alveoli. In another gland, smaller in area, even larger lobules were present.

In the present study on the monkey, most of the male specimens observed were devoid of alveoli. In only a few was there definite lobule formation. Most of the studies on the human male breast have been based upon relatively few specimens. It is suggested, therefore, that the disagreement which still exists in the literature as to the normal occurrence of alveoli in the male may well be the result of inadequate sampling.

## CYCLIC CHANGES

In all mammalian species thus far studied, postnatal development of the mammary gland participates, in its early stages at least, in the general somatic growth process and occurs independently of the gonads. With the onset of puberty and the subsequent establishment of recurrent sexual cycles, further growth and differentiation occur in a manner characteristic of and associated with the type of sexual cycle of each species. Turner (1939) has reviewed the literature on the subject and has considered the postpubertal mammary gland changes related to and dependent upon four types of estrous cycle: (1) continuous estrus (rabbit, ferret); (2) short estrous cycles (rat, mouse); (3) long estrous cycles (guinea pig, heifer, monkey, human); (4) seasonal breeding periods (sheep, goat, hedgehog, fox, squirrel).

In the rabbit no mammary development occurs in the absence of ovulation, the latter being dependent upon the stimulus of copulation. The animals remain in continuous estrus until mating occurs, the mammary glands consisting of extensively branched duct trees with few end buds but no true lobules. After ovulation, under the influence of the corpus luteum the extensive lobular development of pseudopregnancy is attained. A similar sequence of events occurs in the ferret.

The estrous cycles in the rat and mouse vary in duration between 4 and 6 days. Even in this brief period changes in the mammary glands, associated with the various phases of the cycle, have been observed. Sutter (1921) described an increased branching and extension of the duct system of the rat during estrus and postestrus, with gradual regression during diestrus and proestrus. These cyclic changes have not been emphasized by the studies of several subsequent workers, but alterations of a somewhat different character were noted by Astwood, Geschickter, and Rausch (1937). These investigators found a secretory phase, manifested by a dilatation of the ducts and distention of the end buds, occurring at postestrus and continuing into diestrus, with subsequent collapse of these structures at the following proestrus. Studies by Turner and Gomez (1933*a*), Cole (1934), and Wieser (1934) are in general agreement concerning the mammary cycle in the mouse. At estrus there is a proliferation of the duct epithelium with a resultant extension and dilatation of the tubules and the appearance of new end buds, regression occurring during diestrus and proestrus.

Cyclic changes in the guinea pig's mammary gland have been well studied by Hesselberg and Loeb (1916), Loeb and Hesselberg (1917*a*, 1917*b*), and Turner and Gomez (1933*b*). The essential features consist in mitotic proliferation of the glandular epithelium during the first phase of the cycle (up to ovulation), with the formation of many duct buds but no true lobules of alveoli. Proliferation is absent during the luteal phase of the cycle, so that gradual regression of the gland occurs. This is not to be interpreted as an insensitivity of the gland to the secretions of the corpus luteum, but is rather a manifestation of the long latent period required by the guinea pig before mammary response to the secretions of the corpus luteum becomes evident. In the rabbit also it is at least five days before the effect of the corpus luteum on the growth of the mammary gland is seen (Hammond and Marshall, 1914). A somewhat similar situation prevails in the heifer. In this species too the gland undergoes changes corresponding to but lagging behind those occurring in the corpus luteum, the mammary epithelium enlarging during the corpus luteum's development and shriveling during its involution (Hammond, 1927). Intracanalicular secretion also occurs just before heat begins, with resorption during the luteal phase.

In the seasonal breeders the mammary glands undergo no development during anestrum. At the beginning of the breeding season the glands usually consist of only a few branching ducts at the base of the nipple. Duct growth occurs with the onset of estrus, and lobule formation ensues following ovulation.

By analogy with the several species mentioned, one would expect to find cyclic changes in the mammary glands of the primates. That alterations do occur in the human breast in relation to menstruation is strongly suggested by the subjective fullness and increase in size which many women experience before the onset of each monthly flow. In cases of mastodynia, exacerbations typically occur before the menses. The occurrence of cyclic changes in the size of the human breast has been demonstrated objectively by means of measurements of X-ray silhouettes (Reimann and Seabold, 1933) and by volumetric determinations

(Geschickter, 1943). Occasionally a milky secretion appears even in the breasts of virgins at the time of their menses, and in rare instances vicarious menstruation has been observed with periodic molimina and the discharge of bloody fluid from the nipple without uterine bleeding (Pfister, 1901).

In 1922 Rosenburg published a paper purporting to establish a histologic cycle in the human mammary gland. His study was based on material obtained from the autopsy room. No alveoli were found in specimens representing the early interval, the glands consisting merely of ducts and supporting tissue. Correlating the mammary histology with an examination of the ovaries, Rosenburg described a progressive budding of the ducts and rapid proliferation of the epithelial elements with the formation of lobules of alveoli, dating from the time of ovulation and continuing throughout the corpus luteum phase of the cycle. Degenerative changes were observed with the onset of menstruation, and involution of the mammary gland with retrogression of the epithelial structures became more advanced during the postmenstrual period. Soon the alveoli disappeared completely, and during the interval the breast was composed again of ducts of varying sizes but no lobules.

The claims of Rosenburg were sharply criticized by Dieckmann (1925). The latter author rearranged Rosenburg's cases and found it possible to correlate the histologic appearance of the mammary gland with the age of the patient. In so doing he noted that the specimens consisting chiefly of ducts had been obtained from the youngest patients and that progressively more lobular development was found as the ages of the patients increased. Denying, therefore, the existence of an epithelial cycle in the gland, as pictured by Rosenburg, Dieckmann went on to describe a cycle consisting of a premenstrual swelling of the lobules with subsequent shrinkage during the postmenstrual period. These changes were attributed to the formation and resorption of edema fluid within the intralobular stroma (Läppchenoedem).

A large number of studies have appeared both in confirmation and in denial of the observations of Rosenburg and of Dieckmann. In addition, claims have been presented for cyclic cytologic changes in the glandular cells and for cyclic alterations in the degree of cellular infiltration within the stroma. No general agreement has yet been reached, however, concerning the existence or extent of any of the changes described in the human mammary gland. The status of the problem was reviewed in detail in an earlier report (Speert, 1941), and the difficulties attendant upon attempts to study the mammary cycle in the human were pointed out.

No direct study has been undertaken to ascertain the existence and nature of a mammary cycle in the subhuman primates. Heape, in his early studies on the menstrual cycle of the macaque (1897), observed that the nipples became swollen and deeply congested at the time of menstruation. From the study of control mammary glands removed at various times in the menstrual cycle in the course of subsequent experimental studies, no evidence has been found for cyclic changes in the breasts of either the rhesus monkey (Allen, Gardner, and Diddle, 1935) or the baboon, *Papio hamadryas* (Parkes and Zuckerman, 1931).

In the present study 35 biopsies were made of the mammary glands of 8 mature female monkeys which were having normal menstrual cycles. The specimens were obtained from alternate breasts at intervals of about one or two weeks, so that 12 menstrual cycles were represented in the composite material. Six of the cycles were ovulatory, 6 nonovulatory (table 4). In each instance the diagnosis of ovulation was made by Dr. Hartman by means of rectoabdominal palpation. Seven laparotomies were also performed, at which time the diagnosis was checked by inspection of the ovaries. In two instances fertilized ova were recovered. The onset of menstruation was noted by inspection of the daily vaginal lavages. In monkey no. 620 hysterectomy was performed on day 22 of the cycle. Since menstruation naturally failed to occur subsequently in this animal, the ovarian cycle was arbitrarily recorded as of 28 days' duration.

Study of gross mounts of the mammary glands clearly revealed cyclic changes which appeared in a definite temporal relation to the bleeding cycle. The changes to be described occurred, however, only following ovulation. No significant alterations were observed in the breasts during nonovulatory menstrual cycles.

Most striking and consistent of the changes was the enlargement of the lobules which occurred during the premenstruum. During the 10 days or so preceding the onset of menstruation, progressive swelling of the lobules was observed, continuing into the period

TABLE 4

MAMMARY BIOPSY SPECIMENS OBTAINED AT VARIOUS STAGES OF THE MENSTRUAL CYCLE

| Monkey | Cycle | Day of cycle | Remarks |
|---|---|---|---|
| 592..... | Ovulatory | 13 | Laparotomy; ovulation day 12; hysterotomy |
| | | 24 | |
| 593..... | Nonovulatory | 8 | |
| | | 15 | |
| | | 22 | |
| | | 3 | |
| 607..... | Nonovulatory | 8 | Preceding cycle ovulatory; ovulation day 13; laparotomy |
| | | 15 | |
| | | 22 | |
| | | 5 | |
| 612..... | Ovulatory | 24 | Ovulation day 13; laparotomy |
| | | 2 | |
| | Nonovulatory | 10 | |
| | | 17 | |
| | | 1 | |
| | | 11 | |
| 620..... | Ovulatory | 22 | Ovulation day 11; hysterectomy |
| | | 0 | |
| | | 6 | |
| | Nonovulatory | 18 | |
| | | 25 | |
| 627..... | Ovulatory | 3 | |
| | | 11 | |
| | | 25 | |
| | | 6 | |
| 646..... | Ovulatory | 21 | Ovulation day 17; removal of ovary with corpus luteum day 21 |
| | | 4 | |
| | Nonovulatory | 11 | |
| | | 21 | |
| 681..... | Ovulatory | 11 | Laparotomy |
| | | 2 | |
| | | 9 | |
| | Nonovulatory | 17 | |
| | | 24 | |
| | | 30 | |

of the menstrual flow. This lobular enlargement appeared to be due entirely to the dilatation of the individual alveoli, which were clearly discernible even in the gross mounts, giving to the lobules a spongelike appearance (fig. 29, pl. 5). Gradual subsidence of the alveolar distention occurred during the postmenstrual period and early interval, so that by the middle of the subsequent cycle the lobules had completely regressed to what appeared in the mounts as a collapsed state (fig. 30, pl. 5). These changes occurred, in varying degree, in all the ovulatory cycles, but were present in none of the nonovulatory ones.

In 4 animals mammary biopsies were compared during two successive cycles, the first of which was ovulatory and the second nonovulatory. The contrast between the changes in the former and their absence in the latter is well depicted in figures 28 to 31, plate 5 (monkey no. 612). Figure 28 shows the appearance of the gland on day 24 of an ovulatory cycle, 11 days after the occurrence of ovulation (diagnosed by laparotomy). The lobules were already enlarging and the individual alveoli were clearly distinguishable. This cycle was of 33 days' duration. In the biopsy specimen obtained 11 days later, on day 2 of the succeeding cycle, the lobules were swollen to about twice their former size and had taken on a spongy appearance, the dilated alveoli standing out more clearly (fig. 29). Mammary biopsy was repeated on day 10 of this cycle, at which time the subsidence of lobular swelling was apparent (not illustrated). This cycle was nonovulatory (diagnosed by bimanual palpation). By day 17 further regression of the lobules was clearly seen and there was a decided increase in the visible amount of interlobular stroma. The individual alveoli could be made out now only with difficulty (fig. 30). On day 1 of the next cycle, biopsy of the gland revealed essentially the same picture, no increase in size of the lobules having occurred (fig. 31). A striking contrast is presented by figures 29 and 31, which represent approximately the same stage of two successive cycles, the first following upon an ovulatory cycle, the second a nonovulatory one. The lobules of the latter specimen had shrunk to a fraction of their former size.

## ROLE OF THE CORPUS LUTEUM

In the preceding section the occurrence of lobular enlargement of the mammary gland during the premenstruum was shown to be associated with the presence of a corpus luteum, for the cyclic changes described were found in only those cycles during which ovulation had occurred. This finding is in agreement with numerous observations that have been made in several other species, all indicating a causal relation between the corpus luteum and mammary development. This relation has been best studied in the rabbit, beginning with the experiments of Ancel and Bouin in 1911. The mammary glands were

removed from pseudopregnant rabbits at various intervals following sterile coitus and it was found that the extent of glandular development could be correlated with the age of the corpus luteum. Hammond and Marshall (1914) confirmed these findings in the rabbit and described a similar relation in the ferret (1930); in this species also, no mammary growth occurs during estrus, however prolonged, before the formation of the corpus luteum.

Marshall and Halnan (1917) and Turner and Gomez (1934) have shown that the mammary gland of the dog attains complete growth of the lobulo-alveolar system during pseudopregnancy, and this has been attributed to the influence of the corpus luteum. Similar pseudopregnancy development has been demonstrated in the mammary gland of the fox (Rowlands and Parkes, 1935). In the marsupial *Dasyurus* the entire process of growth has been ascribed to the corpus luteum (O'Donoghue, 1911). Hartman (1921, 1923) has also demonstrated mammary hypertrophy during pseudopregnancy in the opossum. The initial growth impetus at least must have been independent of the corpus luteum, since mammary growth was observed to begin before ovulation. This is in contrast with observations in the rabbit and cow, in which species a short latent period follows ovulation before the mammary response to the influence of the corpus luteum is detectable.

The luteinization of the ovaries of mice which is induced by the injection of extracts of sheep anterior pituitary or of human pregnancy urine results in the development of secondary ducts and alveolar buds in the mammary glands, but these mammary effects do not occur in castrated animals (Bradbury, 1932). According to Selye, Collip, and Thomson (1934), persistence of the corpora lutea may be produced in virgin rats by distention of the uterus with paraffin, and the mammary glands of animals so treated undergo a degree of development typical of pseudopregnancy.

A new approach toward the problem was made by Knaus (1930), who studied the effects of withdrawal of the corpus luteum influence. He found that if the corpora lutea were removed from rabbits at the 5th day of pseudopregnancy, distinct involution of the glands could be observed 72 hours later. In agreement with this was the report of Asdell and Salisbury (1933) that no mammary development occurred following ovariectomy in rabbits which had been mated with vasectomized bucks.

Multiple corpora lutea usually occur bilaterally following the induction of ovulation in the rabbit. Interpretations based upon the results of ablation experiments in this species are therefore complicated by the fact that both ovaries were either entirely removed or grossly disturbed. Hence one cannot with absolute certainty attribute the resultant effects to the specific absence of the corpus luteum. It was of interest, therefore, to study the monkey's mammary gland following removal of the ovary containing the current corpus luteum, the other ovary being intact.

Repeated bimanual palpations were performed on monkey no. 646, and on the 5th day after the first ovulation of the current season, day 21 of the menstrual cycle, the ovary containing the corpus luteum was removed. The other ovary, which was left *in situ,* contained follicles but no corpora lutea. Figure 32, plate 6, shows the condition of the mammary gland at this time, with greatly enlarged lobules and distended alveoli. Mammary biopsy was repeated 5 days after operation. In this short time definite shrinkage of the lobules had occurred (fig. 33, pl. 6), although the individual alveoli were still clearly seen. Seven days later another biopsy of the gland revealed further regression of the lobules, as manifested by their smaller size and by an increase in the interlobular stroma. The individual alveoli were, for the most part, no longer distinguishable (fig. 34, pl. 6).

These changes are similar to those observed in the mammary gland during normal ovulatory cycles, except for the more abrupt onset of the retrogressive phase brought about by the surgical removal of the corpus luteum. The view is therefore strengthened that the changes in the macaque mammary gland during the menstrual cycle are directly attributable to the hormonal activity of the corpus luteum.

## NONEFFECT OF HYSTERECTOMY

Prolongation of the life of the corpus luteum can be effected in laboratory rodents by several techniques, including injection of gonadotrophic hormones of pituitary or chorionic origin, administration of estrogens, suckling or other mechanical stimulation of the nipples, and chronic distention of the uterus. To

Leo Loeb goes the credit for first demonstrating that increased longevity accrued to the corpus luteum following hysterectomy in the guinea pig. In this report (1923) and also in a later report (1927), Loeb described the mitotic proliferation and lobular growth, even occasional milk formation, which occurred in the mammary glands of hysterectomized animals, and he attributed this mammary development to the activity of the corpus luteum. Later experiments by Asdell and Hammond (1933), Loeb and Smith (1936), Gillard (1937), and Chu and Lee (1942) extended these observations in part to the rabbit, for it was shown that hysterectomy during pseudopregnancy in this species resulted in a prolongation of the life of the corpora lutea. In contrast with the guinea pig, however, no further mammary development occurred in the rabbit beyond that normally attained during pseudopregnancy.

Observations in other species have failed to demonstrate an effect of hysterectomy on either the corpora lutea or the mammary glands. In the rat, for example, removal of the uterus during any stage of the estrous cycle failed to produce persistence of the corpora lutea (Hechter, Fraenkel, Lev, and Soskin, 1940), and Hartman (1925) found no difference between hysterectomized animals and normal controls in the diestrous involution of the mammary glands of the opossum. Hysterectomy probably has no specific effect on the human ovary, although some gynecologists believe that the impaired circulation to the gonads which results from the operation may produce premature menopausal symptoms in some women.

In the monkey also, removal of the uterus seems to have no effect on the ovaries. Hartman (1932) observed an animal for one year following total hysterectomy. During this period she continued to have normal ovarian cycles, as indicated by the vaginal desquamation, and at autopsy the ovaries were in "an excellent state of preservation." Burford and Diddle (1936) likewise found no impairment of ovarian function as manifested by the vaginal lavage, sex skin, and examination of the ovaries at laparotomy, in 5 hysterectomized macaques which were observed for 121 to 217 days after operation; and histologic sections of the ovaries revealed no increase in follicular atresia. In 2 additional monkeys, hysterectomy following parturition had no demonstrable effect on the resumption of ovarian activity, as indicated by the vaginal desquamation (Van Wagenen and Catchpole, 1941).

Since no observations have been made on the monkey's mammary gland following hysterectomy, we studied monkey no. 620 in some detail for 6 months after the operation, which was performed on the 22d day of the cycle, 11 days after ovulation (Speert, 1942*c*). Mammary biopsies were made at the time of hysterectomy and 5, 11, 23, and 30 days postoperatively. Distinct enlargement of the lobules was observed between the time of operation and the 5th postoperative day, which was the 27th day of the cycle and the time of the expected maximal effect of the corpus luteum on the mammary gland. Photographs of these biopsy specimens appeared in a preliminary report on the cyclic changes in the mammary gland (Speert, 1941). The subsequent biopsy specimens revealed no further development of the gland. On the contrary, rather prompt shrinkage of the lobules occurred, for the following cycle was nonovulatory. This retrogression of the mammary lobules was regarded as a manifestation of the absence of a functional corpus luteum.

Four and one-half months following hysterectomy, laparotomy was repeated and bilateral ovariectomy was performed, together with biopsy of the mammary gland. Mammary biopsy was repeated 45 days later. In the interim moderate diffuse lobular atrophy had set in, as well as the hyperplastic nodules which have been described previously in castrates with intact uteri (Speert, 1940*c*). These castration changes will be referred to in a later section. Hysterectomy, therefore, appeared to have no effect on the monkey's mammary gland, either before or after castration.

## PREGNANCY

With the exception of hypertrophy of the uterus, the changes in the mammary glands are the most striking which occur in mammals as a result of pregnancy. The rapidity of the mammary development during this period and the abruptness with which lactation follows parturition are unequaled, either by the changes in other glands of the body or by the alterations in the mammary gland itself during any other period of similar duration. Because of the obvious functional significance of the gestational growth of

the mammary gland, a large proportion of the studies of this organ have concerned themselves with this phase of its development. Previous reports by others have described the pregnancy changes in the mammary glands of the rat, mouse, bat, rabbit, squirrel, hedgehog, guinea pig, dog, *Dasyurus,* goat, cow, and human.

In each species two types of change have been described. The first, occurring during the first part of pregnancy, consists predominantly in growth (hypertrophy and hyperplasia); the second, characterizing the later stages of gestation, is chiefly one of differentiation (secretory modification of the cells and enlargement of the alveoli). In the smaller animals rather fixed lines have been drawn separating these two stages of development, although the transition is, for the most part, a gradual one. In the rat, for example, proliferation is practically complete by the 13th day, secretory changes appearing from this point to term, 21 days (Roberts, 1921). Similarly in the mouse, hyperplasia of the mammary parenchyma is complete at the end of the 12th day (Turner and Gomez, 1933*a*). In the guinea pig, duct and alveolar development is confined to the interval between the 20th and 35th days of the 63- to 67-day period of gestation (Loeb and Hesselberg, 1917*a;* Turner and Gomez, 1933*b*). Likewise in the goat, proliferation of the lobulo-alveolar system occurs predominantly during the first half of the 150-day pregnancy (Turner and Gomez, 1936), and in cattle mammary growth is complete at about the 5th month, or midway to term (Turner, 1934).

Observations on the human breast during pregnancy are not entirely satisfactory, as was pointed out previously. Yet they have served to indicate the same general tendencies of growth in early pregnancy and differentiation during the later stages that have been demonstrated in other species. Lewis and Geschickter (1934) described the rapid proliferation of the glandular elements in the first third of pregnancy, with maturation of the cells and dilatation of the alveoli occurring chiefly during the latter half of gestation. Grynfeltt (1937) found the maximal development of the galactophores at the end of the 2d and beginning of the 3d month. Rapid formation of ductules in early pregnancy has also been observed by Dawson (1935), who described the casting off of the superficial cells of these newly formed two-layered structures at about the 3d month. This was interpreted as the structural basis for the formation of colostrum, which is usually observed clinically only after the 3d month.

The present studies are the first which have been made of the pregnancy changes in the mammary gland of the monkey. A large number of biopsy specimens were studied, corresponding to very early pregnancies of 11 to 12 days conception age. The range of structural variation encountered among these specimens was extremely wide, as has been described in a previous section. In addition, 25 pairs of mammary glands were on hand, representing later stages of pregnancy up to term (165 days). Observations were made on 5 animals by means of serial biopsies at various stages. By this method it was possible to follow in the same monkey the sequence of changes from early pregnancy to completion of involution, as many as 9 biopsy specimens being obtained from a single animal. A complete series of observations was made on each of the following:

Monkey no. 601. Biopsies on days 33, 68, 97, and 144 (parturition) of pregnancy, and days 3, 17, 51, and 95 postpartum.

Monkey no. 654. Biopsies on days 23, 95, 128, 157, and 161 (parturition) of pregnancy, and days 4, 24, 54, and 87 postpartum.

Monkey no. 690. Biopsies on days 30, 57, and 106 (parturition) of pregnancy, and days 3, 15, 49, and 92 postpartum.

Great individual variation was found among the specimens obtained during the first 2 months of gestation. In some the lobules were well developed, the alveoli were moderately dilated, and mitotic figures were present. In others, representing corresponding stages of early pregnancy, the glandular structures were still poorly developed and did not even equal, in many instances, the development encountered in the glands of some nonpregnant animals during normal menstrual cycles. Despite these variations in the early stages, certain general tendencies of mammary development during pregnancy have emerged from the background of our composite material.

In an examination of the gross mounts (figs. 35–38, pl. 6), little change was apparent between the 1st and 3d months of pregnancy. The lobules were of about the same size, although they had become appreciably denser in appearance (figs. 35, 36). The ducts were also of approximately the same caliber and

thickness. During the 3d month the glands underwent a decided increase in thickness. Great hypertrophy of the lobules occurred, so that in the gross mounts progressively less interlobular stroma remained visible. The alveoli became enlarged to such an extent by the 4th month that they could be distinguished at the periphery of the lobules, even at low magnification (fig. 37). The blood vessels also became more prominent as a result of their increased caliber. Specimens obtained at subsequent stages of pregnancy revealed still further increase in thickness of the gland. The lobules became progressively larger, chiefly because of the dilatation of their alveoli. The individual alveoli were clearly distinguishable at the periphery of the lobules as term was approached, despite the virtual replacement of the interlobular stroma by the enlarging lobules (fig. 38).

The histologic basis for the gross changes observed in the mounts was revealed in microscopic sections of the same specimens (figs. 39–42, pl. 7). The paucity of lobular growth during the 1st month of pregnancy was confirmed. Glands which were poorly developed at the time of conception still showed no better lobular development at the end of a month than was observed in many nonpregnant animals. Histologic sections of such glands after 1 month's gestation showed lobules which were just beginning to take form. These were composed of small numbers of alveoli and were rather sparsely spaced in an undifferentiated connective-tissue stroma (fig. 39). The patent alveoli were formed by moderately large cuboidal cells which contained large, round nuclei and relatively little cytoplasm. Occasional mitotic figures were seen. Through the stroma coursed numerous blood vessels, which supplied the lobules with dilated capillaries filled with red blood cells.

Alveolar proliferation occurred at a gradually increasing rate during the 2d and 3d months. Specimens obtained after 2 months' gestation revealed a relatively slight increase in the size of the lobules. The alveoli and the individual cells appeared somewhat larger and there was a relative increase in the amount of cytoplasm in the latter. Mitoses were slightly more numerous but were still not conspicuous.

The greatest impetus to glandular growth became manifest during the 3d month of pregnancy. Tremendous lobular hypertrophy occurred during this period, with a resultant relative decrease in the interlobular stroma. The increase in size of the lobules by the end of the 3d month was due, in the main, to the rapid multiplication of the alveoli (fig. 40). An increasing number of mitotic figures further attested a more rapid rate of proliferation. The alveolar cells also appeared larger. Superimposed upon this proliferative activity were clear-cut evidences of secretory activity in the mammary gland at this stage of pregnancy. The nuclei were basally located, many cells contained supranuclear vacuoles, and in others secretory processes jutted from the cell margins into the lumina of the alveoli. The majority of the alveoli were more or less distended and contained small amounts of colostrum (fig. 40). This secretion, which was also present in the ducts, presented an amorphous appearance microscopically and gave no evidence of desquamated epithelial cells or leukocytes, which are commonly observed in the colostrum of other species. Secretory activity was well established in most glands by the beginning of the 4th month of pregnancy. Some variation was encountered in this respect, however. In monkey no. 690, for example, there was no evidence of secretion in histologic sections of the mammary gland at 106 days.

Biopsies at subsequent stages of pregnancy up to term revealed a continuation and accentuation of secretory activity. The lobules became progressively larger and the alveoli became increasingly distended with colostrum (figs. 41, 42). The cells and their nuclei increased in size, but as term was approached and the products of secretion distended the alveoli, their walls became thinned out in many places. Mitotic figures continued in evidence in moderate numbers. Vascularity of the lobules became increasingly prominent with the approach of parturition.

During the last trimester of pregnancy many glands contained an increased proportion of eosinophiles in the blood vessels. The significance of this finding is not clear. Eosinophiles have been described in the stroma and among the alveoli in the breasts of the human newborn, and they are known to occur in the adult breast also. It is probable that these cells are present in the mammary glands of other animals as well, for Jeffers (1940) has described them in the bat at the time of parturition.

Many authors who have described the human mammary gland during pregnancy have commented upon the presence in every breast of small lobules which

have failed to undergo the complete development of their neighbors. These have been termed virginal lobules, and their failure to respond to the hormonal stimulation of pregnancy has been attributed to inherent focal deficiencies in the glandular structure. Recalcitrant lobules of this sort were never encountered in the monkey. In every mammary gland at or near term, all lobules were found to have undergone complete and rather uniform development.

The mitotic proliferation of the alveolar cells which is responsible for the rapid growth of the mammary gland in early pregnancy was described as early as 1887 in the glands of dogs, rabbits, cats, rats, and guinea pigs (Bizzozero and Vassale, 1887). In these species mitotic growth is restricted largely to the first half of pregnancy. In the rat, for example, mitoses are rare after the 13th day (Roberts, 1921). In 3 human specimens which Grynfeltt (1936) studied, representing the 4th, 6th, and last months of pregnancy, no mitotic figures were found. Our material, by contrast, revealed an increased incidence of mitotic figures in the mammary gland of the monkey during the second half of gestation. Figure 43, plate 7, shows an example of the frequency of mitoses at term. This biopsy specimen, obtained at 157 days' pregnancy and 4 days before parturition, contained numerous mitotic figures, five of which, including three consecutive mitoses in the wall of an alveolus, are shown in the photograph. The enlargement of the monkey's mammary gland during the later stages of pregnancy is the result of continued mitotic proliferation of the cells as well as dilatation of the alveoli.

Various experimental procedures, such as the transplantation experiments of Ribbert (1898), Basch (1906), and Stricker (1929), the parabiosis experiments of Ernst (1927), the nerve resections of Pfister (1901), and the injection of hormones, have established beyond question the endocrine basis of the mammary development of pregnancy. This concept has been widely accepted for many years; yet the precise hormonal mechanisms responsible for the pregnancy changes in the mammae are still not entirely clear. It is likely that the several factors involved assume different degrees of importance in the different mammals.

Hildebrandt (1904), on the basis of clinical and veterinary observations, was the first to propound the theory that the products of conception produce a substance which at the same time causes growth of the mammary gland and inhibits lactation. By clever analysis of well known clinical observations, Halban (1905) developed this theory further, attributing the mammary development of pregnancy to the influence of the ovaries and placenta, and the inhibition of lactation to the latter organ. This theory has survived to the present day.

The early experiments of Lane-Claypon and Starling (1906), Aschner and Grigoriu (1911), and Biedl and Königstein (1911), based on the injection of fetal extracts into rabbits and guinea pigs, suggested the fetus as the source of the mammotrophic stimuli. Subsequent reinterpretation of this work, however, has indicated the probable incorrectness of their conclusions. Attention was later directed to the ovaries, in particular to the corpus luteum, as the agent responsible for mammary growth during pseudopregnancy and, by implication, during pregnancy. Ancel and Bouin (1911) demonstrated the identity of the mammary changes of pseudopregnancy with those which occur during the first part of pregnancy in the rabbit (phase de développement gravidique). These changes were attributed directly to the influence of the corpora lutea, but were held to be of different origin from the secretory changes of the latter part of pregnancy (phase glandulaire gravidique), which were associated with the so-called myometrial gland (Bouin and Ancel, 1912). The extraovarian origin of the stimulus for mammary development during the latter part of pregnancy in the rabbit was further attested by the failure of attainment of the final phases of development in animals whose corpora lutea were maintained by hysterectomy, repeated sterile copulations, or the injection of anterior pituitary extracts (Asdell, Hammond, and Salisbury, 1933).

Castration can usually be performed with impunity during pregnancy in the guinea pig, so far as the maintenance of the gravid state is concerned. In animals which have continued to term following extirpation of the ovaries, normal mammary development has been observed (Herrick, 1928; Nelson, 1934). In the human also the evidence is quite convincing that the ovaries are not the trophic center for the genital organs, except perhaps during the earliest stages. Halban cited in 1905 several cases of bilateral ovariectomy during pregnancy in which normal mammary development and lactation occurred. Many

such cases have been observed since then, for example, the patient of Young (1941) in whom both ovaries were removed at about the 35th day of gestation. Pregnancy continued uneventfully to term, pregnanediol excretion was within normal limits during the last month, and lactation occurred following parturition.

In the rhesus monkey pregnancy has been undisturbed by bilateral ovariectomy performed at various stages. Hartman (1941) reported an uninterrupted gestation [1] following operation as early as the 25th day, and more recently this experiment was confirmed in 2 additional cases by Corner (personal communication, 1943). As in the human and the guinea pig, mammary development proceeds normally in the monkey in the absence of the ovaries, as indicated by the following observations.

Monkey no. 457 was castrated on the 46th day of pregnancy. Histologic examination of the mammary gland at parturition revealed complete development of the lobulo-alveolar system, including secretory activity and numerous mitotic figures. This picture differed in no essential detail from the histologic pattern encountered at term in animals with intact ovaries.

Monkey no. 466 underwent bilateral ovariectomy on the 31st day of gestation. Mammary development remained unaffected, for examination of the gland at 140 days revealed complete development, comparable in all respects to that normally seen in the mammae of intact animals near term.

Unilateral ovariectomy with removal of the ovary containing the corpus luteum of pregnancy was performed on monkey no. 567 on the 35th day. Abortion occurred on about the 87th day, 52 days after operation. Examination of the mammary gland 10 days later revealed a fully developed and lactating organ.

Recent observations by Dorfman and Van Wagenen (1941) are of interest in this connection. These authors demonstrated an increase in the urinary titers of both estrogens and androgens during pregnancy in the rhesus monkey. Removal of the fetus by abdominal hysterotomy, in addition to castration, was found to have no significant effect on these hormonal levels, if the placenta was left undisturbed. Estrogens and androgens are effective mammotrophic agents, either singly or in combination, as will be demonstrated in a later section. The adrenals have been regarded as the probable source of the increased androgen titer of pregnancy, but the large amounts of estrogens which are produced during this period almost certainly originate in the placenta. This organ is thus indicated as the chief although not the sole source of mammotrophic hormones during pregnancy. The precise role of progesterone is still uncertain, for it has not been possible to recover pregnanediol from the macaque's urine (Marker and Hartman, 1940).

Evidence from other sources has pointed to the placenta as the principal organ responsible for the mammary growth of pregnancy. Gynecomastia is a common finding in patients with testicular tumors which contain chorionic elements. In the comparable condition of chorioepithelioma in the female, full breast development and lactation may occur in the absence of corpora lutea (Fischer, 1909). In pregnant rats (Selye, Collip, and Thomson, 1935*b;* Leonard, 1945) and mice (Newton and Lits, 1938) the mammary glands have been maintained and have continued to develop following castration and removal or destruction of all the fetuses, only the placentas remaining intact. Even after hypophysectomy during pregnancy, mammary growth has continued in a fairly normal fashion in the mouse (Newton and Beck, 1939; Newton and Richardson, 1941; Gardner and Allen, 1942) and guinea pig (Desclin, 1939).

Despite the failure of the monkey's mammary gland to undergo in early pregnancy significant development beyond that which is normally attained during an ovulatory menstrual cycle, rapid involution occurs if the pregnancy is interrupted as early as 1 month after conception. In monkey no. 636 pregnancy was terminated on the 36th day by abdominal hysterotomy. Mammary biopsy at this time revealed well developed lobules of moderately dilated alveoli, and dilated blood vessels coursing through the interlobular stroma (fig. 44, pl. 8). Fourteen days after operation, biopsy of the mammary gland was repeated. Shrinkage of the lobules had occurred, and the blood vessels were now quite inconspicuous (fig. 45, pl. 8). This regression of the gland was comparable to that which was observed following ablation of the corpus luteum in a nonpregnant animal.

When pregnancy was interrupted at somewhat later stages, secretory changes representing a modified form

[1] This animal was found subsequently to have a fragment of ovarian tissue adhering to the uterus. The corpus luteum of pregnancy, however, had been removed in its entirety, as was proved by serial sections.

of lactation appeared before the onset of involution. Abdominal hysterotomy was performed on monkey no. 680 on the 60th day of pregnancy, and the products of gestation were removed. The histologic picture of the mammary gland at this time is shown in figure 46, plate 8. The lobules were well developed and the alveoli were somewhat dilated, but there was no evidence of secretory activity. Two days after the operative abortion, mammary biopsy was repeated (fig. 47, pl. 8). Secretory changes were now unmistakably present in the cells as evidenced by marked vacuolization, the alveoli were distended to several times their former size, and the products of secretion could be seen in the lumina of the alveoli. Secretion occurs in the mammary glands of the guinea pig following abortion during the second half but not the first half of pregnancy (Loeb and Hesselberg, 1917*b*). In the rabbit, lactation follows Caesarean section after about the 15th day of pregnancy (Bayliss and Starling, 1906); and in the mouse, hysterectomy as early as the 11th day of pregnancy is followed by milk formation within 48 hours (Bradbury, 1932).

These observations bring to the fore the old problem concerning the mechanism by which lactation is held in abeyance during pregnancy. Meites and Turner (1942) recently examined the different views which have been held on this subject and concluded that progesterone overrides the lactogen-producing effects (on the pituitary) of estrogen during pregnancy. Even in cases where lactation is established, it is a well known clinical and veterinary fact that a supervening pregnancy results in a diminution of milk production. Gaines and Davidson (1926) have expressed this phenomenon by the mathematical formula $di/dp = be^{kp}$, where $i$ represents the decrease in yield, $p$ the time from conception, and $b$ and $k$ are constants. Gaines (1914) has also shown that the transfusion of blood from a pregnant into a lactating goat results in a temporary inhibition of the secretion of milk.

We have had the opportunity to study the histologic picture in the mammary gland of a pregnant monkey shortly after the cessation of nursing activity by this animal. Monkey no. 117 was pregnant, near term, when received into the colony on April 12, and was still lactating as a result of a previous pregnancy. Although milk was expressed from her breasts on April 16, she refused to nurse her offspring. On May 8, about 26 days after weaning, Caesarean section was performed, the animal was killed, and the mammary glands were obtained for study. Histologic sections (fig. 48, pl. 8) presented a rather bizarre appearance that was not quite duplicated in any of the other specimens. In many of the lobules lactation was still in progress: the alveoli were large and irregular, with many of the interalveolar septa ruptured; the cells appeared to be actively secreting, and milk was present within the alveoli. In other lobules early involutionary changes could be seen, with desquamated sheets of alveolar cells lying free within the distended alveoli. In still other lobules, and even in different regions of the same lobules, involution was well advanced: secretory activity had ceased, the alveoli were small, some even collapsed, the cells were small and the nuclei pyknotic, and considerable interalveolar connective tissue had returned and was infiltrated with moderate numbers of round cells. All these changes can be seen in the single microscopic field shown in figure 48. Involution had obviously been modified, but not prevented, by the coexisting pregnancy.

## LACTATION

The remarkable increase in the secretory activity of the mammary glands which occurs after parturition and finds overt expression in the production of milk represents only an accentuation of processes which were initiated in the mammae as early as the middle of pregnancy. When the inhibitory effect of the placenta is removed, increased amounts of lactogenic hormone become mobilized, and in parturient women this substance can be recovered in the urine beginning on the 3d or 4th postpartum day (Hoffmann, 1936), synchronously with the establishment of full-scale lactation. Although milk secretion may set in normally at birth in hypophysectomized animals, sustained lactation occurs only in the presence of a functioning pituitary gland (Selye, Collip, and Thomson, 1933) or adequate substitutional therapy.

The secretion of milk begins within the first 2 days after parturition in the monkey. The mammary glands, which previously lay flat against the chest wall, rapidly enlarge, and when lactation is at its

height, the milk-filled breasts hang in large folds below the costal margins (fig. 11, pl. 2).

The general histologic features of lactation are common to all mammals. We have examined the mammae of several monkeys at various periods of lactation. The lobules were large and the alveoli greatly distended, for the most part, with a faintly staining, granular secretion (milk). Very little stroma was present in the glands. In many places the interalveolar septa were ruptured and the free ends projected into the coalesced alveolar lumina. The septa contained numerous small blood vessels, a few connective-tissue cells, and occasional leukocytes. Eosinophiles appeared prominently among the latter. Michaelis (1898) noted numerous eosinophiles in the interstitial tissue of the mammary gland of the postpartum guinea pig, and more recently others have described increased numbers of eosinophiles in the interalveolar and interlobular connective tissue of the lactating albino rat (Emmel, Weatherford, and Streicher, 1926) and bat (Jeffers, 1940). The former authors expressed agreement with the idea of Rauber (1879) that leukocytes contribute a definite cellular constituent to the composition of milk. In all our specimens the cells which lined distended alveoli were flattened out. In regions where the alveoli were not distended, the cells appeared larger and elongated, and contained abundant vacuolated cytoplasm. The nuclei were large and contained large, single, distinct nucleoli, and granular chromatin. The nuclei tended to be located basally, but in many places they bulged into the lumen of the alveolus. At the height of lactation evidence of secretory activity could be seen in the epithelium lining the small ducts as well as the alveoli. Duct secretion has also been described in many other species, including the cow, cat, dog, guinea pig, and rabbit (Richter, 1928).

The nature of milk secretion has long been the subject of wide differences of opinion. Virchow (1862) regarded the process as a physiologic type of fatty degeneration, the milk globules being set free by the disintegrating epithelial cells. Langer (1852), on the other hand, maintained that death of epithelial cells played no part in lactation; he regarded the process as purely secretory. Rauber emphasized the importance of the leukocytes. These, he held, invaded the alveoli and there underwent degeneration and eventual transformation into milk. Subsequent authors have taken one view or the other, or have assumed intermediate positions or combined various aspects of the different theories.

According to those who have regarded the secretion of milk as a purely vital activity, no further increase in the actual number of glandular cells occurs after parturition. Indeed, their inability to demonstrate cellular proliferation in lactating glands has been one of the points of evidence raised in support of their theory. The larger number of investigators, however, have recognized the need for cellular replacement during the course of lactation. Guillebeau (1916), for example, basing his conclusions on histological considerations and on mathematical calculations of the milk constituents, pointed out the necessity for replacement of gland cells during lactation in the cow and goat.

Mitoses still occur in the mammary gland shortly after parturition. They have been observed in the guinea pig, cat, rabbit, rat, and cow; and we have seen mitotic figures during the early days of lactation in the monkey. They then became rare, however, and soon they could not be demonstrated at all. This is in accord with most of the observations on other species, although a few workers claim to have demonstrated mitotic figures in the mammae at later stages of lactation. Having failed to find mitoses in an examination of hundreds of microscopic preparations of lactating glands, Nissen in 1886 suggested the possibility of direct cell division. Divergent views concerning this possibility were expressed in the literature for many succeeding years, various authors either affirming or denying the occurrence of amitotic nuclear division in the lactating glands of different species.

Amitosis occasionally takes place in cells which have no genetic value, such as those of the ovarian follicular epithelium, placenta, and outer layers of the epidermis. When direct nuclear division does occur, it is rarely if ever accompanied by division of the cell body (Conklin, 1917). Binucleate cells are thus produced as a result of amitosis. Such cells have been observed in the lactating mammary glands of the guinea pig, cat, dog, rabbit, mouse, bat, and human, even in very thin sections. Double and lobulated nuclei have likewise been encountered in the lactating glands of our rhesus monkeys (fig. 49, pl. 8). These nuclei had the same general internal structure as single nuclei. Ami-

tosis is not a true divisional phenomenon, but serves merely to distribute the nuclear material through a greater region of the cell. Increased nuclear surface thereby results, a factor which may be of significance in the synthetic and secretory activity of the cell during lactation.

## INVOLUTION

The rise and decline of milk secretion which occur at the beginning and end of lactation have been regarded as a type of growth and senescence which has the unique property of being renewed following every pregnancy (Brody, Turner, and Ragsdale, 1924). When a certain stage of lactation is reached, the secretion of milk begins to wane, the rate of decline remaining constant until involution is complete (Brody, Ragsdale, and Turner, 1923). Several factors are involved in this process. It has been suggested that milk secretion is limited by a chemical reaction, the speed of which is determined by the concentration of a limiting substance, but the exact nature of this hypothetical substance has never been demonstrated. Of paramount importance are the hormonal factors associated with lactation. The role of the pituitary has already been mentioned, and abundant evidence has indicated the importance of the adrenals also. Halban (1905) compared the puerperal involution of the mammary glands of non-nursing women to the atrophy which follows castration, attributing both to the absence of the protective and regulatory influence of the ovaries. Ernst (1925) attempted to establish a parallelism between puerperal and postmenstrual changes in the breasts. By and large, results of experimental efforts to demonstrate an effect of the ovaries on the postpartum involution of the mammary glands have not been impressive. Kuramitsu and Loeb (1921) were only able to demonstrate a less intense leukocytic invasion of the glands of rats and guinea pigs following castration. Williams (1942*b*) more recently found that neither castration nor the injection of estrone, progesterone, and testosterone, either singly or in various combinations, altered the course of mammary involution in mice whose litters were removed on the 10th postpartum day. Although puerperal involution of the mammary glands has not been studied in castrated primates, it is unlikely that their ovaries play a significant role in this process, except perhaps during the later stages, since ovarian function is at a very low ebb following parturition in man and monkey.

Morphologically, certain similarities have been noted in the involution which occurs in the mammary glands of various species following parturition or weaning. Important differences occur only in the rate of involution and the length of time required for the glands to return to their prepregnant state. In the mouse, regression is complete 5 days after parturition (Williams, 1942*b*) and 13 days after weaning (Cole, 1934). Involution has been well studied in the rat (Myers and Myers, 1921; Maeder, 1922), and the glands were found to have returned approximately to their resting (virginal) state by the end of the 2d or 3d week after weaning. Involution was found to be almost complete in a dog 40 days after parturition (Turner and Gomez, 1934), complete in a cat 80 days after weaning (Turner and DeMoss, 1934), and complete in a guinea pig 100 days following removal of the young (Turner and Gomez, 1933*b*). Complete mammary involution occurred in a goat within 5½ months after the suspension of milking (Turner and Reineke, 1936). In each of these species the lobulo-alveolar system underwent complete atrophy and only the duct system remained. The exact duration of the involutional changes following parturition or lactation in the human has not been determined, but Deaver and McFarland (1917) have stated that they may extend over months or even years when not interrupted by recurrent pregnancy.

The present studies on involution in the monkey are based upon the mammary glands of 22 animals at various stages from the onset of lactation to 127 days after weaning, and in non-nursing monkeys, to 109 days postpartum. In many instances only one specimen was obtained from an animal, but the most instructive material resulted from the serial mammary biopsies on the same animal at various stages of involution. This was accomplished in 8 cases, the most complete series being obtained from the following:

Monkeys nos. 601, 654, and 690 (see section on pregnancy).

Monkey no. 614. Stillbirth on 150th day of gestation. Mammary biopsies on days 9, 25, 36, 66, and 109 postpartum.

Monkey no. 652. Baby weaned 112 days after parturition. Mammary biopsies on days 0, 13, 28, 44, 78, and 108 after weaning.

Monkey no. 665. Baby weaned 42 days after parturition. Mammary biopsies on days 0, 13, 28, 44, 78, and 127 after weaning.

Postpartum and postlactational involution of the mammary glands proceeded at different rates in different animals. In addition, not all parts of each gland presented the same histologic picture, for involution often progressed at a different rate in each lobule. The following description is therefore based on a typical or average case. Special features of involution and important variations will be given separate mention.

Gross mounts of mammary specimens from nonnursing animals showed the lobules in a state of engorgement for approximately 2 weeks. During this period the alveoli and ducts were distended with stagnant secretory products. During the 2d week involutionary changes became evident: the lobules began to decrease in size and the interlobular stroma reappeared. Figure 50, plate 9, shows a typical gland on the 9th postpartum day. Despite the beginning regression of the lobules, the ducts were still distended, even bulging in places, with their retained contents. During the next 2 weeks involution progressed rapidly and the lobules returned to a fraction of their former size. The ducts, however, were still dilated at the end of this period, their diameters often exceeding those of the lobules. Subsequent changes progressed at a relatively slow rate, as judged by the appearance of the gross mounts. By the end of 3½ months involution was complete. Both the lobules and the ducts had returned to a delicate, atrophic state which frequently resembled the castrate condition (fig. 51, pl. 9). Mammary atrophy of this degree, however, did not occur in every case. The return of ovarian function following parturition is subject to considerable variation. If the ovaries are slow to resume control of the secondary sex organs, and the uterus remains small and puerperal amenorrhea persists, then mammary involution continues unaffected. On the other hand, menstruation may occur relatively soon after parturition, indicating an early resumption of ovarian activity. When this occurs, mammary involution is interrupted or growth is renewed, depending upon the condition of the gland and the intensity of ovarian stimulation.

A description of the histologic changes during involution properly begins with the lactating gland. As described in the preceding section, the lactating lobules were large and the alveoli were distended and separated by thin septa which were ruptured in many places. The cells were actively secreting and the products of their secretion could be seen in the alveolar lumina. The histologic appearance of such a gland is shown in figure 52, plate 9, which represents a biopsy specimen obtained from monkey no. 654 at the time of weaning on the 4th postpartum day.

The rapidity of the involutionary changes during the first 3 weeks is clearly shown in a biopsy specimen from the same animal 20 days later (fig. 53, pl. 9). The lobules and their component alveoli were distinctly smaller and there was a corresponding increase in the amount of loose interlobular connective tissue. Much of the intra-alveolar secretion had disappeared, but secretory vacuoles were still present in most of the cells. Great variation was noted in the size of the nuclei; most, however, were considerably smaller than in the lactating gland. They were oval in shape and took a deep stain. A moderate, diffuse cellular infiltration, composed chiefly of round cells, was present in the lobular stroma, with denser accumulations in some regions than in others.

During the next month further decrease occurred in the amount of glandular parenchyma. At the end of this period the lobules were still smaller and the alveoli fewer. Most of the latter were collapsed; those that still remained patent had only small lumina. Few of the cells contained vacuoles, and the nuclei were pyknotic. Increased numbers of round cells were present in the interalveolar stroma. The amount and density of the connective tissue had increased, both within and between the lobules. Particularly striking was the increase in connective tissue about the ducts (fig. 54, pl. 9).

The involutionary changes which occurred during the 3d month represented a continuation of the processes already described. The lobules were greatly reduced in size, so that they consisted of only a few small alveoli with collapsed walls. The latter were made up of shrunken, inactive cells. Scattered round cells persisted throughout the stroma, but in greatly decreased numbers. A further increase was noted in the amount of dense connective tissue, especially surrounding the ducts (fig. 55, pl. 9).

In contrast with the rather uniform development of the mammary gland which occurred during pregnancy, involution was an irregular process. Thus one might find that in one of two neighboring lobules, even after fairly prolonged periods of involution, marked retrogressive changes had occurred, whereas in the other the alveoli were still distended with retained secretion. Such a varied histologic picture might even be observed in different parts of the same lobule. This irregularity of involution is well shown in figure 56, plate 10, which represents a biopsy specimen on the 49th postpartum day. In some of the lobules the alveoli were completely collapsed and a dense cellular infiltration was present. In other regions the alveoli were greatly dilated and secretion was still present within the lumina. Hesselberg and Loeb (1937*a*, 1937*b*), describing the retrogressing mammary gland of the guinea pig, have also called attention to the persistence of areas in which the alveoli have the characteristics of the secreting gland. In the human, McFarland (1922) has even described the so-called residual lactation alveoli, structures which failed to involute because of obstruction to the outlets of the ducts and alveoli. This was held to be the basis for subsequent cyst formation in the breast. The irregularity of retrogression and the persistence of dilated alveoli with retained secretion in the monkey's mammary gland were only temporary aberrations in the involutionary process. When involution was complete, all parenchymal structures had returned to their prepregnant state.

At least two mechanisms were responsible for the reduction in the amount of glandular substance. The rapid decline in vascularity which followed the cessation of lactation caused atrophy of the parenchymal elements, with shrinkage of the alveoli and resorption of the cells. This may be regarded as an atrophy of disuse. Following the rapid death of some of the alveolar cells, large numbers of them sloughed into the lumina. Here these desquamated cells could be seen lying singly or in sheets within both the alveoli (fig. 57, pl. 10) and the ducts (fig. 58, pl. 10). Masses of duct epithelium were lost in a similar fashion, as can be seen in figure 58. Liquefaction and absorption of these cells was probably aided by the leukocytes which migrated into the lumina.

Despite the fact that its precise significance has not been established, leukocytic invasion of the lobules constituted one of the constant and prominent factors in mammary involution. The cellular infiltrate was composed chiefly of round cells, but polymorphonuclear leukocytes and large mononuclear cells could also be demonstrated. These cells appeared in the interalveolar stroma within the first 2 weeks of involution, but did not usually attain their maximal concentration until the 2d month. As with the other phases of involution, considerable variation was encountered with respect to the time and height of the peak of the cellular infiltration, its duration, and its distribution through the mammary lobules. A typical cellular infiltration is shown in figure 59, plate 10, a biopsy specimen obtained 44 days after weaning.

The foregoing description of mammary involution is based upon animals which did not nurse their offspring or in which nursing was interrupted soon after parturition. This description is also applicable qualitatively to animals whose young were weaned after 3 months or more of suckling. Study of the mammary glands of the latter group resulted in the distinct impression, however, that their involution progressed at a somewhat more rapid rate. This is contrary to recent observations by Williams (1942*b*), who found that the postpartum involution which occurred in the mammary glands of the mouse in the complete absence of suckling was more rapid than the involution which occurred after varying periods of lactation.

When placental function is disturbed, as manifested by fetal death in utero, mammary involution may begin before parturition occurs. Monkey no. 449 aborted a macerated fetus on the 101st day of pregnancy. Examination of the maternal mammary gland at this time revealed well established involutionary processes. Monkey no. 690 aborted its fetus on the 106th day of pregnancy, fetal death having been caused within the preceding 3 days by the injection of a foreign substance (Thorotrast) into the amniotic sac. Biopsy of the mammary gland revealed a lactating organ with no signs of involution. In a biopsy specimen obtained 2 weeks later, a histologic picture comparable to that in no. 449 at parturition was observed. The inference seemed justified that placental function had been disturbed in the latter animal approximately 2 weeks before emptying of the uterus occurred, and that mammary involution had progressed during the interim.

Typical involutionary changes were observed in the

mammary glands of animals following the interruption of pregnancy at earlier stages. In monkey no. 680, for example, abdominal hysterotomy was performed on the 80th day, and in monkey no. 636 on the 36th day. Mammary biopsy was repeated 17 days after operation in the former and 14 days after operation in the latter animal. In both instances definite signs of involution were present. The lobules were smaller than at operation, the alveoli were collapsed, an increased amount of connective tissue and a cellular infiltration were present in the stroma, and desquamated epithelial cells lay free in the ducts. Secretory activity, which was present 3 days postoperatively in no. 680, had largely subsided by the 17th day.

## EFFECTS OF CASTRATION

The importance of the ovaries for mammary development is firmly established, but the changes which result from removal of the ovaries are but poorly understood. Little attention has been given to the effects of castration on the mammary gland. Mammary atrophy did not occur in rats (Turner and Schultze, 1931) and guinea pigs (Ebhardt, 1928) following ablation of the ovaries. Observations on the human are scant and contradictory. Alterthum (1899) detected no changes in the breasts, nipples, or areolas of 18 young women after castration. Keppler (1891), on the other hand, found that in 15 cases the nipples and breasts grew significantly smaller (less than one-half their former size) and the areolas became lighter in color after bilateral ovariectomy. In a clinical study of 116 patients, Pfister (1898) observed breast changes in 50 per cent after castration. Atrophy was noted in about 60 per cent of those patients who experienced breast changes, whereas in the remaining 40 per cent the breasts increased in size. In none of these cases, however, were the glands examined histologically.

The only observations on the mammary glands of castrated monkeys are those of Aberle (1934*a*). She compared the glands of 4 animals whose ovaries had been removed 7 days to 6 months previously with those of 5 controls. There was no significant difference in mammary area between the two groups.

Our material includes 155 mammary specimens which were obtained either for biopsy or at autopsy from 85 castrated mature female animals at intervals ranging up to more than 4 years after castration. The large majority of the specimens were from monkeys which were treated with various hormonal preparations subsequent to removal of the ovaries. A few animals were castrated during pregnancy. Many remained untreated. In order to study systematically the effects of castration itself upon the mammary glands, serial biopsy specimens were obtained from 6 animals at various intervals following surgical removal of the ovaries. On these 6, a total of 21 biopsies were performed, beginning at the time of operation and extending through the following 2½ months as follows:

| *Monkey no.* | *Biopsy, days after castration* |
|---|---|
| 592 | 0, 10, 30, 58 |
| 620 | 0, 45 |
| 623 | 0, 20, 50 |
| 660 | 0, 14, 29, 62, 76 |
| 673 | 0, 37 |
| 687 | 0, 14, 29, 62, 76 |

The structural changes which were observed in the mammary glands after castration were of two general types, atrophic and proliferative. These occurred concurrently in the same glands.

### Atrophic Changes

The progress of postcastration atrophy was observed in both the gross mounts and the histologic sections of the biopsy specimens. A typical series is shown in figures 60 to 67, plate 11, which represent mammary specimens obtained from monkey no. 592 at various stages after ovariectomy. On January 1, 1940, castration and biopsy of the mammary gland were performed. The well developed mammary lobules were separated by only small amounts of connective tissue (figs. 60, 64). Definite shrinkage of the lobules occurred within 10 days (figs. 61, 65). Biopsies were repeated on the 30th and 58th days after castration. These specimens revealed a progressive diminution in size of the lobules, with a corresponding increase in the relative amount of interlobular stroma (figs. 62, 66; 63, 67). Microscopically, the alveoli were fewer and smaller and their walls gradually collapsed. The alveolar cells likewise diminished in size and their constituent nuclei became pyknotic.

In some cases, such as that just described, the out-

standing feature of the postcastration changes was the epithelial atrophy. In others this was less striking, attention being drawn rather to the increased amount and density of the interlobular connective tissue. This change is well shown in biopsy specimens obtained from monkey no. 623 at the time of castration (fig. 68, pl. 12) and 50 days later (fig. 69, pl. 12). The lobular atrophy, although distinct, was not as great as that observed in monkey no. 592. The stromal changes, on the other hand, scarcely discernible in the photographs of the latter, were unmistakably clear in monkey no. 623. This degree of sclerosis was manifested even in the fresh state by an increased toughness of the tissue and by its resistance to dissection.

### Proliferative Changes

Concurrently with this generalized epithelial atrophy and stromal sclerosis, localized hyperplastic nodules developed throughout the mammary glands of mature castrated females. These nodules consisted of well circumscribed islets of alveolar tissue which stood out in sharp contrast from the surrounding regions of the gland (fig. 70, pl. 12). The development and structure of these tumors have been described in a previous paper (Speert, 1940*c*) and will be referred to only briefly here.

Nodule formation occurred as early as 29 days after removal of the ovaries, and definitive tumors were found as long as 18 months after spaying. This was the longest postcastration interval represented among the glands from untreated animals, but nodules persisted as long as 4 years after castration in the mammary glands of other animals which received various hormonal injections. The tumors appeared grossly as white, opaque, dense, round or oval discoid masses which lay in the plane of the mammary gland and ranged from 1 to 6 mm. in diameter. Great variation was observed in the number of nodules. In some glands only a solitary tumor was present, whereas others contained 20 or 30, irregularly spaced among the remaining atrophic lobules. Histologic sections revealed their adenomatous nature, the microscopic appearance bearing a close resemblance to that of normal mammary parenchyma. Mitotic figures were abundant and secretory vacuoles were usually present in the alveolar cells. Tumors appeared in the glands of 18 castrates which received no hormonal treatment after operation. Mammary nodules were usually absent in a series of 57 other castrated animals, 2 of which were pregnant at the time of operation and the remainder of which were treated with various hormones after removal of the ovaries. The hormones included various natural estrogens, diethylstilbestrol, testosterone and its esters, progesterone, and desoxycorticosterone acetate. Each of these was effective in preventing formation of the nodules when treatment in adequate dosage was begun during the first month after castration. Once the nodules had appeared, however, hormonal treatment was ineffectual in causing their regression or in modifying either their gross or microscopic structure.

Of the 43 specimens which contained nodules, all but 6 were from castrated animals. These 6 exceptions challenge the specificity of the nodular change in relation to absence of the ovaries. Five of the 6 noncastrated animals, however, gave evidence of having undergone at least a period of seriously altered ovarian structure or function (partial hypophysectomy, large ovarian cysts, amenorrhea). The mammary glands of these animals were indistinguishable from those of the castrates (fig. 71, pl. 12).

It is tempting to compare the present observations on the spayed monkey with certain pathologic changes which occur with great frequency in the breasts of women around and beyond the menopausal age. Lobular irregularities can be demonstrated microscopically in more than one-third of clinically normal breasts during this period (Geschickter, 1943). Tietze (1904), von Saar (1907), and Dietrich (1926) regarded proliferative and cystadenomatous changes as a manifestation of ovarian failure and almost physiologic for old age. Similar proliferative changes have been observed in the apocrine glands of the axilla in 40 per cent of women over 51. These glands, like the mammae, are intimately dependent upon gonadal function (Buschke, 1933).

Mammary tumors have also been observed in untreated ovariectomized mice (Fekete, Woolley, and Little, 1941; Gardner, 1941*b*). These tumors were associated with nodular hyperplasia of the adrenal cortex and occurred in animals of a high cancer strain. In the rhesus monkey, by contrast, spontaneous mam-

mary cancer has not been observed, nor has it been produced experimentally with large amounts of estrogenic hormone (see section on "Effects of hormones").

The pathogenesis of mammary tumors in the monkey's breast following castration is not clear. Estrogenic and gonadotrophic substances persist in the blood and urine of women after the natural menopause or surgical removal of the ovaries (Frank, Goldberger, and Salmon, 1936; Fluhmann and Murphy, 1939; Walter, Geist, and Salmon, 1940), and the urinary titer of 17-ketosteroids increases after bilateral oöphorectomy (Ross, Hamblen, Cuyler, and Baptist, 1941). In a strain of mice bearing adrenal tumors, castration caused a threefold increase in the excretion of estrogens (Dorfman and Gardner, 1944). Whether these phenomena have any significance for our observations on the monkey is uncertain, nor do they explain the focal occurrence of proliferative activity in the presence of generalized atrophy of the mammary gland. The prevention of postcastration tumors of the macaque breast by ovarian and other steroidal hormones may be related to the ability of these compounds to prevent castration changes in the hypophysis (Clarke, Albert, and Selye, 1942).

## EFFECTS OF HORMONES

During the past decade, since many hormones have been available in chemically pure form, it has been possible to correlate certain mammary changes, both normal and abnormal, with specific groups of chemical compounds. Foremost among the mammotrophic hormones are those of ovarian origin. The biologic characteristics of the female mammary gland during sexual development and maturity are governed principally by the ovaries. Functional ovarian grafts in young or castrated guinea pigs (Steinach, 1912; Athias, 1915; Sand, 1918; Smelser, 1933), rats (Engle, 1929), and mice (Gardner, 1935) stimulate mammary growth. The atrophic changes which follow ovariectomy in the monkey, described in an earlier part of this report, are equally striking.

The lengthy paper which Fellner published in 1913 reported the first successful effort to demonstrate the presence in the ovaries of a factor which causes growth of the mammae. Fellner obtained a positive mammary response by injecting rabbits subcutaneously or intraperitoneally with extracts of ovaries which contained corpora lutea. Several investigators confirmed these observations, using crude ovarian extracts, but little progress was made toward identification of the specific ovarian hormone which caused growth of the mammary glands until Allen and Doisy (1923) prepared an active ovarian extract from the liquor folliculi and developed a biological assay method for its standardization. When Doisy, Veler, and Thayer (1930) later crystallized a pure estrogen from human pregnancy urine, other workers, in rapid succession, determined the chemical structure of the natural estrogens as well as the androgens, progestogens, and some of the adrenocortical hormones. All these compounds were found to belong to the general group of cyclopentenophenanthrenes.

### Estrogens

As a result, renewed interest arose in the mammo-ovarian relation and the possibilities for studying its quantitative aspects. Numerous experiments demonstrated the growth-promoting effects of ovarian, and later synthetic, estrogens on the mammary glands of the rat, mouse, cat, dog, opossum, guinea pig, rabbit, goat, cow, monkey, baboon, and human. Although the results were uniformly positive, the type and degree of the growth response which the estrogens elicited varied from species to species. In the majority the effects were limited rather sharply to an increase in extent and arborization of the duct system.

The guinea pig is the best-known example of an animal in which complete lobular development results from estrogens alone. Limited development of lobules has been produced in some other species, such as the rat and rabbit, by appropriate treatment with large amounts of estrogen. Most animals, however, require additional treatment with a progestogen for the attainment of full lobular growth.

In the human, enlargement of the breasts and nipples and proliferation of the mammary ducts commonly occur after the administration of natural or synthetic estrogens to males (Dunn, 1940; Solomon, 1941; Scarff and Smith, 1942; Kahle, Ogden, and Getzoff, 1942; Pearlman and Pincus, 1943; Klein and Newman, 1944; Moore, Wattenberg, and Rose, 1945), castrated females (Werner and Collier, 1933; Werner,

1935; Guldberg, 1938), women with inadequate breast development (MacBryde, Freedman, Loeffel, and Castrodale, 1940; Lisser, 1940), pseudohermaphrodites (Bettinger, 1944), children (Woodruff and Te Linde, 1942), and newborn infants (Speert, unpublished). The histologic changes in the few mammary biopsies which have been performed before and after estrogenic treatment of women who required plastic breast operations (Hoffmann, 1939; Bishop, Boycott, and Zuckerman, 1939) were not sufficiently clear-cut or consistent to permit conclusions as to the nature of the response.

The effects of estrogen on the mammary gland of the monkey were first studied by Allen (1927, 1928*a*, 1928*b*), who compared gross mounts of the breasts after treatment with control mounts from the same animal. Allen reported an increase in the area of the gland, a dilatation and branching of the ducts, and a multiplication of the alveoli. In these experiments relatively small amounts of estrogen were used (maximum average daily dose 50 r.u.) and the periods of treatment were short (maximum 24 days). Moreover, the published illustrations were not convincing, for only a small part of the control gland was shown for comparison with the entire gland after treatment. Subsequent experiments by Allen and others, however, have clearly demonstrated the potent mammotrophic effects of estrogens, in adequate dosage, in the monkey. Turner and Allen (1933), for example, found extensive lobular development in a male monkey after 65 days' treatment with rather large amounts of estrone, and Gardner and Van Wagenen (1938) obtained complete glandular development in young males following treatment for 22 to 30 weeks with smaller doses of estrogen. Using intermediate amounts, Van Wagenen (1935) produced orderly growth of the mammary glands of immature male animals, including development of the terminal knobs or end buds. Experiments by Folley, Guthkelch, and Zuckerman (1939) have suggested that the female monkey's breast is more responsive to estrone than is that of the male.

The present observations on the effects of estrogens on the monkey's mammary gland include experiments on 1 male and 24 females, large and small, which were treated with various preparations for periods ranging from 12 days to 2½ years. Fifteen of the females were surgical castrates. The results of treatment are summarized in table 5, a positive growth response being indicated by +, no detectable response by −. The various natural estrogens to which the monkey's breast was responsive included estrone, Amniotin (Squibb), estradiol, and estradiol benzoate. The test substances were administered subcutaneously, sometimes injected in oil, sometimes implanted in pellet form.

The degree of response depended on two major factors, namely, dosage and duration of treatment. Estrone or Amniotin, in amounts ranging from 25 to 100 rat units per day, was without effect in castrates during periods of treatment up to 45 days. One such animal (no. 140), which received Amniotin injections of 100 r.u. daily, showed definite mammary growth in 50 days. The latter conditions of dosage and time appear to approach the minimum for detectable mammary response to estrogens. As might be expected, smaller amounts of estrogen were effective when the animal's ovaries were present. Thus, no. 693 showed a positive response to 50 r.u. of estradiol benzoate daily at the end of 1 month.

The rate of secretion of estrone by the ovaries of young adult rhesus monkeys has been estimated by Corner (1940) at about 200 International Units daily. This represents roughly one-half of what we have found to be the minimal effective dose for mammary growth over a period of 50 days. It is to be expected, however, that a larger than physiologic amount would be required for the production of so prompt a response. Our findings and Corner's estimate may therefore be regarded as compatible and, to a degree, mutually supporting.

The experiments of Nelson (1936) and of Gardner and Van Wagenen (1938) are in general agreement with our observations. The former reported distinct growth of ducts and lobules in 3 monkeys after 4 to 7 months' treatment with 100 to 200 r.u. of estrogen daily for 20 and 63 days respectively. Folley, Guthkelch, and Zuckerman (1939) claimed growth in the glands of 4 out of 5 male monkeys which received estrone for 6 to 15 days, in amounts ranging from 500 to 50,000 r.u. per day, but a critical examination of the published data and photographs fails to reveal definite evidence of mammary growth in these animals. We have found, however, that even in so short a period as 2 weeks pronounced hyperplasia of the ducts can be elicited if the estrogenic level is high enough (fig. 76, pl. 13). Heroic experiments of this

TABLE 5

MONKEYS TREATED WITH ESTROGENS

| Monkey | Sex | Weight (gm.) | Treatment | | | Mammary response |
|---|---|---|---|---|---|---|
| | | | Estrogen | Dose | Duration | |
| 140 | F(c)* | 4120 | Amniotin | 100 r.u./d. | 50 d. | + |
| 392 | F(c) | 4370 | Amniotin | 1000 I.U./d. | 32 d. | + |
| 470 | F(c) | Immature | Estrone | 25 r.u./d. | 14 d. | − |
| 560 | F | 2580 | Estradiol | 50,000 I.U./d. | 70 d. | + |
| 626 | F(c) | 6020 | Amniotin | 25–100 r.u./d. | 45 d. | − |
| 660 | F(c) | 4320 | Stilbestrol | 0.1–1 mg./d. | 30 d. | − |
| | | | Estradiol benzoate | 50–150 r.u./d. | 25 d. | + |
| 687 | F(c) | 4120 | Stilbestrol | 0.5–2 mg./d. | 30 d. | − |
| | | | Estradiol benzoate | 50 r.u./d. | 25 d. | + |
| 693 | F | 4400 | Estradiol benzoate | 50 r.u./d. | 30 d. | + |
| C15 | M | 5970 | Estrone | 2000 I.U./2 d. | 70 d. | + |
| G20 | F(c) | 3200 | Estrone | Two 3-mg. pellets/week | 78 d. | + |
| | | | Amniotin | 10,000 I.U./2 d. | | |
| G21 | F | 3470 | Same | Same | 106 d. | + |
| | | | Same | Same | 8 mo. | + |
| | | | Same | Same | 11 mo. | + |
| | | | Same | Same | 14 mo. | + |
| G22 | F | 3940 | Same | Same | 5 mo. | + |
| G23 | F(c) | 2890 | Same | Same | 85 d. | + |
| G24 | F(c) | 3970 | Same | Same | 76 d. | + |
| | | | Same | Same | 10 mo. | + |
| | | | Same | Same | 12 mo. | + |
| | | | Same | Same | 14 mo. | + |
| | | | Same | Same | 30 mo. | + |
| G25 | F(c) | 2670 | Same | Same | 36 d. | + |
| | | | Same | Same | 11 mo. | + |
| | | | Same | Same | 17 mo. | + |
| | | | Same | Same | 29 mo. | + |
| G27 | F | 2180 | Same | Same | 2 mo. | + |
| G29 | F(c) | .... | Same | Same | 10 mo. | + |
| | | | Same | Same | 23 mo. | + |
| G33 | F(c) | 3360 | Same | Same | 15 mo. | + |
| G34 | F(c) | 3180 | Same | Same | 33 d. | + |
| G35 | F(c) | .... | Same | Same | 17 mo. | + |
| G37 | F(c) | 3640 | Same | Same | 16 mo. | + |
| G38 | F(c) | 3100 | Same | Same | 3 mo. | + |
| | | | Same | Same | 5 mo. | + |
| G32 | F | 2320 | Same | Same | 8 mo. | + |
| | | | Same | Same | 19 mo. | + |
| G26 | F | 1910 | Estradiol | 95 mg. subcutaneously | 12 d. | + |
| | | | Estradiol | Five 3-mg. pellets/week | | |
| | | | Estradiol | 30,000 I.U./2 d. | | |
| G31 | F | 1300 | Same | Same | 18 d. | + |

* F(c) signifies castrated female.

sort lend themselves poorly to an evaluation of the specific action of a hormone upon an end organ, for such large amounts of estrogen (113 mg. estradiol) induced profound metabolic disturbances in the test animal, including generalized inhibition of growth (Hartman, Geschickter, and Speert, 1941). We have found 1 month to be a reasonable period for testing mammotrophic activity. Moderate amounts of a test substance may fail to produce their effects within a shorter time.

The monkey's breast responded qualitatively in the same manner to the various estrogens, as well as to the

other hormones which exerted a trophic effect upon it, if certain histological changes produced by testosterone and by very large amounts of estrogen, to be described later, are excepted. The mammary response to hormonal stimulation consisted in an accentuation of the normal growth processes: hyperplasia, dilatation, extension, and branching of the ducts, and growth of the lobules by multiplication and dilatation of the alveoli. Secretory activity was not a normal concomitant of the growth process.

The effects of 1 month's treatment with moderate amounts of estrogen (50 r.u. estradiol benzoate daily, ovaries intact) are shown in figures 72 to 75, plate 13, which represent typical biopsy specimens before and after treatment. Photographs of the whole mounts show the increase in optical density of the gland, growth of the lobules with a corresponding decrease in the interlobular stroma, and even the dilatation of the individual alveoli (figs. 72, 73). Histologic sections of the same specimens confirmed these changes (figs. 74, 75).

With large doses of estrogen (10,000 I.U. Amniotin every other day and two 3-mg. pellets estrone a week), mammary growth increased in tempo and magnitude. Many mitotic figures were found among all the epithelial elements, and the rapidly proliferating duct cells produced a stratification of the duct lining 6 to 10 cells thick within a month. This resulted in an initial narrowing of the duct lumina. Under sufficiently intense estrogenic stimulation, stratification proceeded to the point of virtual occlusion of the lumina (fig. 76). Beginning dilatation of the ducts and formation of lobules became evident after about 2 months' treatment.

As the lobules grew larger the ducts continued to dilate. In some places the dilatation was so pronounced as to suggest cysts when the ducts were viewed in histologic section. With continuing development, however, both the stratification and the dilatation subsided and the ducts reverted to narrow, one-layered structures, nestled inconspicuously among the massive lobules of alveoli (figs. 77, 78, pl. 13).

Lobular growth continued for varying periods, some as long as 10 months. During this developmental phase, microscopic examination revealed a high degree of vascularity of the lobules, the interalveolar spaces being crowded with dilated blood capillaries. Mitotic figures were frequently seen among the cuboidal epithelial cells. In some cases lobular edema was present and round cells infiltrated the loose connective tissue which separated the alveoli. The lobules were maintained by continued estrogenic treatment, but the edema subsided, the alveoli became packed together more closely, mitotic figures grew scarce, and blood capillaries were less conspicuous. The monkey's mammary gland apparently can be maintained thus in a state of complete development for an indefinite period, for no evidence of glandular regression was observed in animals under continuous treatment for periods up to 30 months.

With 5 mg. (50,000 I.U.) of estradiol daily, complete glandular development was produced in a prepubertal female in only 70 days (figs. 79, 80, pl. 14). Similar results were obtained in a prepubertal castrate in 85 days (figs. 81, 82, pl. 14). It is interesting that no increase occurred in the area of the mammary gland despite its high degree of internal development (figs. 81, 82). Whereas moderate amounts of estrogen cause a growth in mammary area (Gardner and Van Wagenen, 1938), large amounts of the hormone seem to exercise an inhibitory effect on glandular extension (Gardner, 1941*c*).

Moderate lobular development occurred in an immature male monkey (no. C15) which received 2000 I.U. of estrone every other day for 70 days.

In contrast with the natural estrogens, stilbestrol had little or no effect on the monkey's breast in the two experiments in which it was tested. Each animal was injected with stilbestrol in oil daily for 1 month, no. 660 receiving increasing amounts from 0.1 to 1 mg. per day, and no. 687 receiving 0.5 to 2 mg. per day. The monkey's uterus is known to respond to stilbestrol (Engle and Crafts, 1939), but the amounts used in our experiments may have been too small to produce an effect upon the mammary gland. Dodds, Lawson, and Noble (1938) and Felding and Møller-Christensen (1939) have both observed in the guinea pig that stilbestrol is only one-fifth as active as estrone in causing growth of mammary tissue. When no. 660, immediately after its failure to respond to stilbestrol, was injected with estradiol benzoate in amounts increasing from 50 to 150 r.u. per day, distinct evidence of growth was found after 25 days' treatment.

Because of its capacity to induce growth of the mammary gland, the ovary has long been associated with cancer of this organ. Three decades ago Lathrop

and Loeb (1916) found that castration resulted in a striking reduction in the incidence of mammary carcinoma in a strain of mice highly susceptible to the disease. Sixteen years elapsed, however, before a successful attempt was made to produce cancer of the mammary gland with estrogenic hormones (Lacassagne, 1932). Males (which are normally resistant to the disease) of a tumor-bearing strain of mice developed mammary carcinoma after treatment with large amounts of estrogen. These observations were subsequently confirmed and extended by numerous investigators (see review by Loeb, 1940). Mammary cancer has also been produced in rats by intensive estrogenic treatment (Geschickter, 1939).

In addition to hormonal influences, two other factors are important for the development of mammary cancer; namely, heredity and the so-called milk factor. Their relative importance probably varies among different species. Experimental work to date has been predominantly on mice. Isolated clinical case reports (Allaben and Owen, 1939; Auchincloss and Haagensen, 1940; Parsons and McCall, 1941; Foote and Stewart, 1945) have suggested a possible carcinogenic action of estrogens on the human breast. Although the amounts of estrogen required for the production of malignant changes in the mammary glands of rodents are proportionately many times greater than the doses ordinarily used in the treatment of human patients, such case reports, together with the results of laboratory studies, have naturally brought into question the complete safety of prolonged estrogenic therapy in women.

The monkeys which underwent long-term treatment with very large amounts of estrogenic hormone were of special interest. Fourteen females ranging in weight from 2180 to 3970 gm., 10 of which were surgical castrates, were treated by subcutaneous implantation of two 3-mg. pellets of estrone weekly and injections of 10,000 I.U. of Amniotin in oil every other day. Thirty-two mammary specimens were obtained, by repeated biopsy and terminal autopsy, after periods of treatment up to 30 months.

The experiments were begun by Dr. Geschickter with a view to determining the possibility of producing mammary cancer in the rhesus monkey. All specimens were therefore studied in great detail, and pathological changes were carefully sought. Suffice it to say that no mammary tumors nor any change suggesting malignancy were found, either macroscopically or microscopically. Folley, Guthkelch, and Zuckerman (1939) and Engle, Krakower, and Haagensen (1943) have likewise reported failure of estrogens to produce mammary cancer in monkeys, even when older animals were used. These experiments emphasize the essentiality of the hereditary (species) factor in carcinogenesis. Spontaneous malignant tumors are rare in subhuman primates, and there has been no recorded case of cancer of the mammary gland in a monkey.

Concurrently with the orderly development of the mammae, benign metaplastic changes were observed in the breasts of 5 of our highly estrogenized monkeys. At various periods after the 10th week of treatment small islets of atypical pale epithelial cells appeared in the glands, irregularly spaced among the normal mammary lobules and in the linings of the ducts. Because of their pale staining characteristics, these groups of cells contrasted sharply with the normal mammary epithelium (fig. 83, pl. 14). The pale cells were cuboidal or polygonal and 2 or 3 times as large as the normal mammary cells. The faintly eosinophilic cytoplasm appeared clear at low-power magnification, but at high power it was found to be finely granular, sometimes vacuolated. The large, round nuclei, usually centrally located, were hypochromatic also and contained prominent nucleoli.

Pale epithelium was observed in 4 of the 10 castrated monkeys (40 per cent), but in only 1 of the 4 animals with ovaries intact (25 per cent). Although this difference must be evaluated with consideration for the smallness of the numbers, evidence from another source attests the possible importance of the absence of the ovaries for the genesis of pale epithelium. Metaplastic changes resembling those which we have observed were produced in the mammary glands of male and castrated female rats treated with large amounts of estrogen, whereas such changes rarely occurred in the glands of intact females which were similarly treated (Herold and Effkeman, 1936).

Pale epithelium was first described in the human breast, in the linings of mammary cysts, by Borst in 1904. Since then these atypical cells have been referred to in the literature as "blasse Zellen," "idrosadenoid epithelium," "cellules claires," "oxyphile Epithelien," "eosinophile Epithelien," "helle rosige Epithelien," and "pale epithelium." Opinions concerning their origin, biologic significance, and clinical importance

have been conflicting (Speert, 1942*b*). Pale cells are frequently found in mammary cysts in cases of chronic cystic mastitis as a result of metaplasia of normal mammary epithelium. Our findings support the view that pale epithelium is not necessarily associated with mammary carcinogenesis.

## Progestogens

Significant amounts of progesterone are formed only during pregnancy or, in the nonpregnant animal, during the luteal phase of the ovarian cycle. In either circumstance progesterone acts and produces its specific structural and functional effects on reproductive organs which have been sensitized previously to estrogen. The effects of progesterone on the estrogen-primed primate uterus have been well studied. Little is known of its effects on the primate breast. Indeed, there is no recorded experiment showing the combined action of estrogen and progesterone on the monkey's mammary gland.

The two hormones were administered to monkey no. 425, a castrated female weighing 3100 gm., in a manner designed to simulate a normal ovarian cycle. For 12 days Amniotin (100 r.u.) was injected daily. Beginning on the 8th day and continuing for 18 consecutive days, 1 cc. of progestin (70 gm. equiv.) was also injected. Mammary biopsies were performed before and after treatment. Sections of the biopsy specimens revealed distinct growth of the mammary gland, with enlargement of the lobules and multiplication of the alveoli (figs. 84, 85, pl. 15). These changes were accentuated beyond those which normally occur in association with the menstrual cycle in intact animals. In explanation of this difference two factors may be mentioned. First, the mammary gland of no. 425, having undergone postcastration atrophy, would naturally reveal more readily an increase in size of the lobules or number of alveoli. Second, it is likely that the injected hormones were somewhat in excess of the amounts normally formed by the ovaries of the intact monkey.

In addition to the parenchymal growth brought about by combined treatment with estrogen and progesterone, interesting stromal changes were also observed in the mammary gland. A moderate increase occurred in the number and dilatation of the blood vessels; but more striking was the generalized change in the character of the connective tissue. Its compact, cellular matrix gave way to a loose, fibrillar stroma containing large quantities of interstitial fluid. This phenomenon was observed also in a few castrates which were treated with testosterone propionate (see below), but not in animals which were treated with other hormones.

Positive mammary responses were also obtained in 2 castrated females which were treated with anhydro-hydroxy-progesterone (ethinyl testosterone) after receiving injections of 100 r.u. of Amniotin daily for 8 days. No. 613 was injected with 5 mg. of the compound daily for 18 days. No. 620 received 20 mg. daily by mouth for 15 days. It usually takes a considerably larger amount of anhydro-hydroxy-progesterone by mouth than of progesterone by injection to produce a characteristic progestogenic effect.

When physiologic or even moderately increased amounts of progesterone are administered to castrated animals, the characteristic effects of the hormone usually fail to appear. They require, in addition to progesterone, preliminary or concurrent treatment with an estrogen. Thus, Fredrikson (1939) found the mammary glands of ovariectomized rabbits unresponsive to progesterone alone, nor was any effect produced in the glands of castrated rats so treated (Astwood, Geschickter, and Rausch, 1937). When large enough amounts of the hormone were injected, however, characteristic development of the secondary sex organs, including the mammary glands, was effected in rats (Selye, 1940; Reece and Bivens, 1942), mice (Mixner and Turner, 1942), and monkeys (Hartman and Speert, 1941).

Crystalline progesterone in oil, in daily doses of from 5 to 20 mg., was injected subcutaneously into 3 female monkeys [2] which had been castrated for periods ranging from 37 days to 4 years. Biopsies of the mammary gland were performed before and after treatment, duration of which extended up to 32 days, as shown in table 6. Mammary growth occurred in each case. The lobules increased in size (figs. 86, 87, pl. 15) as their constituent alveoli dilated and multiplied (figs. 88, 89, pl. 15). The individual alveolar cells grew larger and mitotic figures appeared among them. These changes were greatest in the

[2] Monkey no. 673 was used, at different times, in 2 separate experiments, making 4 experiments all together.

monkey which received the largest amount of progesterone; namely, no. 673 after 32 days' treatment with 20 mg. of the hormone daily. Similar but less pronounced growth effects were observed in each of the other 3 experiments. The stromal changes mentioned in an earlier paragraph also occurred in each of the animals receiving progesterone only, the connective tissue losing its denseness and cellularity and becoming loose and edematous (figs. 88, 89).

TABLE 6

MONKEYS TREATED WITH PROGESTERONE

| MONKEY | SEX | CASTRATED | WEIGHT (GM.) | TREATMENT | | MAMMARY RESPONSE |
|---|---|---|---|---|---|---|
| | | | | Daily dose | Days | |
| 623..... | F(c) | 50 days | 4250 | 5 mg. | 32 | + |
| 673..... | F(c) | 37 days | 4950 | 20 mg. | 32 | + |
| 673..... | F(c) | 8½ mos. | .... | 10 mg. | 19 | + |
| 145C.... | F(c) | 4 yrs. | 7330 | 20 mg. | 27 | + |

These experiments clearly demonstrate that crystalline progesterone, in adequate dosage, is capable of inducing its mammotrophic effects in monkeys which have been castrated as long as 4 years, without preliminary priming with estrogen. As in castrated mice, however, which required approximately 6 times as much progesterone for alveolar growth as when estrone was injected simultaneously (Mixner and Turner, 1942), the amounts of progesterone used in the present experiments were well beyond the physiologic range of the monkey.

## GONADOTROPHINS

The administration of gonadotrophic hormones results in development of the mammary glands in most laboratory animals. Extracts of pituitaries, of human pregnancy urine and blood, and of pregnant mare serum produce varying degrees of mammary growth in the rat, mouse, rabbit, and other species. Differences of opinion exist as to the presence of specific mammotrophic hormones in the pituitary (see section on "Mode of action of hormones"). Certain extracts of this gland are capable of stimulating mammary development in castrated animals. However, purified extracts of pituitary, blood, or urine which contain gonadotrophic hormones to the exclusion of others require the ovaries for the mediation of their effects on the mammary glands. They are ineffectual in castrates.

The sensitivity of the ovaries of animals such as the rat, mouse, rabbit, dog, cat, horse, and ewe to gonadotrophic hormones provides a ready explanation of the importance of the gonads in this reaction. Follicular development and luteinization, which result from gonadotrophic stimulation, produce sources of estrogen and progesterone. These, in turn, cause growth of the mammae.

Species differ greatly in the response of their ovaries to gonadotrophins. The rhesus monkey is notoriously recalcitrant, especially to attempts to cause it to ovulate (Hartman, 1938, 1942). It was therefore of interest to examine the mammary glands of monkeys which were treated in an effort to induce ovulation, and to correlate the resulting mammary effects with the changes which occurred in the ovaries.

Biopsies of the mammary gland were performed before and after treatment of 3 nonovulating, amenorrheic adult females with various gonadotrophic preparations. These experiments were included in a previous report by Hartman (1942).

Monkey no. 699 received subcutaneous injections of a mixture of 1 unit of FSH (Jensen) [3] and 10 units of Follutein (Squibb) [3] daily for 16 days. For the next 19 days treatment consisted of injections of Gonadogen (Upjohn),[4] beginning with 10 units daily, decreasing to 3 units daily, and then increasing to 50 units daily, a total of 141 units. Although this treatment produced no palpable effect on the ovaries, uterine bleeding occurred during the course of the FSH-Follutein injections. This may be taken as an indication of ovarian activity. Biopsies of the mammary gland revealed a poorly developed system of lobules consisting of but a few end buds at the beginning of treatment (fig. 90, pl. 16). After treatment the end buds were somewhat increased in number and had undergone slight branching and pronounced dilatation (fig. 91, pl. 16). On the whole, however, mammary stimulation was slight after 35 days' treatment with these gonadotrophins.

Monkey no. 673 was injected with FSH, Follutein, and Gonadogen intermittently over a period of 4

[3] Extract of human pregnancy urine.
[4] Extract of pregnant mare serum.

months. Treatment began with 5 units of FSH and 50 units of Follutein daily for 22 days. After a lapse of 6 days 5 units of Gonadogen were injected daily for 11 days, then 10 units daily for 3 days, and finally 30 units intravenously, a total of 115 units in 16 days. One month later another course of injections of FSH and Follutein, 1 unit of the former and 10 units of the latter daily, was given for 16 days. A second series of Gonadogen injections was given 2 months later, consisting of 10 units daily for 5 days. Ovarian stimulation was indicated by uterine bleeding during the first course of treatment with Gonadogen and by swelling of the sex skin and bleeding from the uterus after the second series of injections with FSH and Follutein; but again there was no palpable change in the condition of the ovaries. Good mammary growth occurred during the several courses of treatment. A biopsy specimen at the beginning of the gonadotrophin injections showed the mammary gland to be distinctly atrophic, as was consistent with the amenorrheic condition of the animal. Only narrow ducts and a few shrunken lobules were present (fig. 92, pl. 16). Following treatment the gland was moderately well developed, numerous lobules of alveoli having sprouted from the proliferating end buds. A moderate degree of dilatation of the ducts had also occurred (fig. 93, pl. 16).

Monkey no. 667 had been amenorrheic for 79 days when treatment was begun. Five units of FSH and 50 units of Follutein were injected daily for 22 days. No apparent stimulation of the ovaries resulted, so far as could be determined by palpation; the sex skin remained unaffected; the uterus failed to increase in size; and no bleeding occurred during or following treatment. Mammary biopsies revealed no change in the gland.

The gonadotrophic hormones obtained from human pregnancy urine and pregnant mare serum are capable of stimulating the ovaries of nonovulating, amenorrheic adult monkeys under certain conditions (Hartman, 1938, 1942). The present experiments indicate that such ovaries, even in the absence of palpable change in them, can cause development of the mammary glands. The occurrence of mammary growth could be correlated with other evidence of ovarian stimulation. Since our observations were limited to 3 animals, however, the role of the gonadotrophins is merely suggested. Ovarian activity in the rhesus monkey is subject to many vagaries. Experiments based on the action of gonadotrophic hormones must therefore be interpreted with reservations. Despite the fact that the animals were nonovulating and amenorrheic, one cannot be certain that the observed results of ovarian activity were not caused by spontaneous reawakening of the dormant gonads rather than by their stimulation by the test hormones.

## Androgens

Although it has been established beyond doubt that estrogens and progestogens are the hormones primarily responsible for the normal proliferation of mammary epithelium, extension of the duct system, and development of lobules of alveoli, these effects can be achieved experimentally by other hormones as well. The mammotrophic action of the androgens, or male sex hormones, for example, has been demonstrated in several species. Testosterone, testosterone propionate, androstenediol, and androstenedione produced duct growth and alveolar development in castrated and intact rats of both sexes (Nelson and Gallagher, 1936; Selye, McEuen, and Collip, 1936; Astwood, Geschickter, and Rausch, 1937; Noble, 1939; Reece and Mixner, 1939; Laqueur and Fluhmann, 1942; Laqueur, 1943). Testosterone propionate, 17-methyl testosterone, and $\Delta_5$-trans-androstenediol stimulated growth of the mammary ducts in guinea pigs (Bottomley and Folley, 1938); and the first of these compounds together with diethylstilbestrol dipropionate, caused udder and teat growth in sterile heifers (Reece, 1943). In the human, gynecomastia resulted in several male patients who underwent therapy with methyl testosterone (McCullagh and Rossmiller, 1941).

The effects of androgens on the monkey's breast have been studied by Folley, Guthkelch, and Zuckerman (1939) and by Van Wagenen and Folley (1939). The former authors treated male animals with testosterone propionate and reported dilatation of the ducts and formation of alveoli but no significant increase in the area of the glands. The latter authors administered various androgens to castrated preadolescent females and compared the mammary gland after treatment with the control gland removed beforehand. They too observed dilatation of the ducts, without

extension or arborization, but denied the capacity of testosterone propionate to produce alveolar development unless alveoli were already present in the gland. Both groups called attention to irregular heaping of the epithelium, resembling intraductal papillomas, in an occasional animal treated with this hormone.

The present experiments comprise three groups of monkeys: 10 animals treated with androgens alone (testosterone, testosterone propionate, methyl testosterone, and androstenediol) (table 7); 3 castrated females treated concurrently with an estrogen and an androgen (table 8); and 4 castrated females treated with an androgen after priming with an estrogen (table 9). Mammary biopsies were performed before and after treatment. Details of the experiments are shown in the tables. A positive growth response is indicated by +, no response by −.

TABLE 7

MONKEYS TREATED WITH ANDROGENS

| Monkey | Sex | Weight (gm.) | Treatment | | | Mammary response |
|---|---|---|---|---|---|---|
| | | | Androgen | Dose | Days | |
| 591 | F | 4590 | Testosterone propionate | 50 mg./d. | 16 | + |
| | | | No treatment | | 28 | Atrophy |
| 681 | F(c) | 5040 | Testosterone propionate | 20 mg./d. | 30 | + |
| | | | No treatment | | 12 | − |
| 692 | F(c) | 3800 | Testosterone propionate | 20 mg./d. | 31 | + |
| | | | No treatment | | 10 | − |
| 620 | F | 4860 | Testosterone propionate | 15 mg./d. | 20 | + |
| 427 | F(c) | 4110 | Testosterone propionate | 10 mg./d. | 25 | + |
| | | | No treatment | | 35 | Atrophy |
| 463* | F(c) | 5540 | Testosterone | 10 mg./d. | 13 | + |
| 591 | F(c) | 4590 | Testosterone propionate | 2 mg./d. (by mouth) | 20 | + |
| M8 | M(c) | .... | Testosterone | 5 mg./d. | 15 | + |
| 472 | F(c) | 3350 | Androstenediol | 2½ mg./2 d. | 33 | − |
| 626† | F(c) | 6020 | Methyl testosterone | 10–20 mg./d. (by mouth) | 7 | − |

* Primed with Amniotin (100 r.u./d.) for 5 days before testosterone injections were begun; i.e., before control biopsy.
† Primed with Amniotin (increasing from 25 to 100 r.u./d.) for 42 days before treatment with methyl testosterone was begun; i.e., before control biopsy.

In the group receiving androgens alone (table 7), 5 females were treated by subcutaneous injection of testosterone propionate, in amounts ranging from 10 to 50 mg. daily, for periods from 16 to 31 days. Three of the animals were castrates, 2 intact. As a result of treatment, distinct enlargement of the lobules and dilatation of the alveoli occurred in each case (figs. 94–97, pl. 16). No dilatation of the ducts, however, and little evidence of secretory activity were to be found. Stromal changes similar to the edema of the interlobular connective tissue described in the progesterone-treated animals were also present in a few among this group (figs. 96, 97).

A castrated male (no. M8) and a castrated female (no. 463) were injected for 2 weeks with 5 and 10 mg. of testosterone daily, respectively. Mammary growth occurred in both cases but to a lesser extent than resulted from treatment with testosterone propionate. In the male, moderate dilatation of the ducts was observed, as well as lobular development (figs. 98, 99, pl. 17).

Androstenediol, 2½ mg. every other day for 33 days, and methyl testosterone, 10 to 20 mg. by mouth daily for a week, were each without effect in 2 female castrates.

The lobulo-alveolar development which resulted from androgenic treatment was quickly reversible upon withdrawal of the hormone. In no. 591 and no. 427 mammary biopsies 28 and 35 days, respectively, after cessation of treatment revealed gross atrophy of the lobules with cellular infiltration of the stroma, collapse of the alveoli, and shrinkage of the cells (figs. 100, 101, pl. 17). These changes probably occurred rapidly after the 2d week of hormonal withdrawal, for they were not found in biopsy specimens obtained from no. 692 and no. 681, 10 and 12 days respectively after treatment was stopped.

Various combinations of an estrogen and an androgen were administered by subcutaneous injection to 3 castrated females for short periods, 10 to 21 days (table 8). A positive mammary response was obtained in each instance. In no. 256 and no. 603 the amount of estrogen was probably inadequate, in itself, to cause detectable mammary development. It seems reasonable, therefore, to attribute the observed response either to the androgen alone or to a synergistic action of androgen and estrogen.

When castrated females were treated with androgens after being primed with Amniotin, a positive growth response was obtained in 3 out of 4 experiments (table 9). Methyl testosterone, by mouth, was ineffective. But in the 3 other animals distinct mammary growth resulted from the sequential action, first of Amniotin, and then of testosterone or one of its esters.

In some respects androgens are antagonistic to estrogens. For example, testosterone and its propionic ester abolished the vaginal response to estrogen in the rat (Courrier and Cohen-Solal, 1937) and human (Shorr, Papanicolaou, and Stimmel, 1938). In other respects androgens are distinctly estrogenic. In the monkey, for instance, testosterone causes a reddening of the sex skin (Hartman, 1937). Present findings confirm the mammotrophic action of androgens in the monkey. When these are administered in conjunction with estrogens, either concurrently or sequentially, no antagonism appears. On the contrary, our observations suggest that estrogenic priming potentiates the effect of androgens, with a resultant synergistic action on the mammary gland. Monkey no. 602 furnished a good example of this synergism. At the beginning of treatment the mammary gland of this castrated female was completely atrophic (figs. 102, 104, pl. 17). Amniotin (100 r.u. daily) was injected for 12 days, following which 25 r.u. of Amniotin and 50 mg. of testosterone were injected daily for the next 15 days. Mammary biopsy at the conclusion

TABLE 8

MONKEYS TREATED CONCURRENTLY WITH ESTROGENS AND ANDROGENS

| MONKEY | SEX | WEIGHT (GM.) | TREATMENT | | | | | MAMMARY RESPONSE |
|---|---|---|---|---|---|---|---|---|
| | | | Estrogen | Daily dose | Androgen | Daily dose | Days | |
| 256* . . . | F(c) | 5270 | Amniotin | 50 r.u. | Testosterone | 25 mg. | 10 | + |
| 597. . . . | F(c) | 3520 | Estrone | 1½-mg. pellet | Methyl testosterone | Six 3-mg. pellets | 18 | + |
| | | | Estradiol propionate | 1 mg. | | | | |
| 603. . . . | F(c) | 4400 | Amniotin | 25 r.u. | Methyl testosterone | 10 mg. | 21 | + |

* Primed with Amniotin (100 r.u./d.) for 8 days before combined treatment was begun; i.e., before control biopsy.

TABLE 9

MONKEYS TREATED SEQUENTIALLY WITH ESTROGEN AND ANDROGENS

| MONKEY | SEX | WEIGHT (GM.) | TREATMENT | | | MAMMARY RESPONSE |
|---|---|---|---|---|---|---|
| | | | Hormones | Daily dose | Days | |
| 602. . . . . | F(c) | 4510 | Amniotin | 100 r.u. | 12 | |
| | | | Amniotin | 25 r.u. | 15 | + |
| | | | Testosterone | 50 mg. | | |
| 585. . . . . | F(c) | 4350 | Amniotin | 50 r.u. | 10 | |
| | | | Testosterone propionate | 10 mg. | 18 | + |
| 584. . . . . | F(c) | 5850 | Amniotin | 100 r.u. | 5 | |
| | | | Testosterone dipropionate | 5 mg. | 17 | + |
| 613. . . . . | F(c) | 4380 | Amniotin | 25–100 r.u. | 19 | |
| | | | Methyl testosterone | 10 mg. (by mouth) | 9 | – |

of this treatment showed a remarkable degree of lobular development, with dilatation of the ducts and alveoli and many mitotic figures (figs. 103, 105, pl. 17). This rapid developmental change was far greater than that experienced by no. 591 in response to 50 mg. of testosterone propionate daily for 16 days without estrogen.

Intraductal and intra-alveolar epithelial proliferation, resulting in microscopic papillomas, occurred in the mammary glands of these same two (no. 602 and no. 591) but in none of the others among the entire group which received androgens (figs. 105, 106, pl. 17). From these 2 animals it is concluded that epithelial heaping can be induced by testosterone or its propionate, either with or without estrogen, and in a castrated or intact monkey. This type of hyperplasia occurred also in a minority of the monkeys treated with testosterone propionate by Folley, Guthkelch, and Zuckerman (1939) and by Van Wagenen and Folley (1939). Its significance, especially in relation to proliferative mastopathies in women, and the possibility of producing or preventing it with other hormones, remain to be clarified.

In a few instances we observed a clear secretion and dilated ducts in sections of the breasts of monkeys treated with androgens (fig. 105). These phenomena, however, were inconstant. More often the ducts were not dilated nor did they contain any visible secretion. We must therefore disagree with the statement of Van Wagenen and Folley that "in the rhesus monkey the most consistent and striking effect of androgens (particularly with testosterone propionate) on the mammary gland consists of the induction of a secretory condition of the epithelial cells, which in turn leads to dilation of the ducts." These authors injected their animals only once or twice a week rather than every day. A secretory reaction is commonly encountered in the mammary gland during the first few days after the cessation of treatment with a mammotrophic hormone. It is suggested, therefore, that the observed secretion may have resulted, at least in part, from a succession of hormonal withdrawals.

Androgenic substances are excreted in significant quantities in the urine of normal women as well as men (Gallagher and others, 1937; Callow and Callow, 1939; Werner, 1941; Fraser and others, 1941). These compounds are presumed to be of adrenal cortical origin, since they are contained in essentially the same titers in the urine of ovariectomized and normal women (Hamblen and others, 1939; Hirschman, 1939; Furuhjelm, 1940). The commingling of "masculine" and "feminine" hormones in the urine has been referred to by Frank (1940) as "chemical hermaphroditism." Andric and gynic factors are not necessarily opposite in character, but rather covariant, forming what Draper (1944) has called a "mosaic of androgyny." When testosterone propionate is injected into human female castrates, a rise occurs in the level of estrogens in the urine (Nathanson and Towne, 1939). Zondek (1934) has even suggested that endogenous androgens are the source of the large quantities of estrogenic hormone which are normally excreted in the urine of the stallion. Considering further the various effects which testosterone and its related compounds are capable of producing in female animals, such as enlargement of the vagina and uterus (Korenchevsky and Hall, 1937) and progestational changes in the endometrium, it becomes apparent that the term "male sex hormones," as applied to these compounds, is a misnomer.

## Desoxycorticosterone

Close chemical relation exists between the sex hormones, the 17-ketosteroids, and the corticosteroids. A well known member of the last group is desoxycorticosterone, which is widely used clinically in the treatment of adrenal insufficiency. The most important physiologic action of this compound is on the body's metabolism of salt and water. Experiments have shown that it also affects the secondary sex organs of laboratory animals, including the mouse, rat, rabbit, cat, guinea pig, chicken, and monkey, simulating estrogens in certain of its actions, progesterone in others, and androgens in still others.

Desoxycorticosterone acetate exerts an estrogenic effect on the vaginal smear of postmenopausal women (Salmon, 1939). In castrated monkeys it increases the vaginal desquamation, causes reddening and swelling of the sex skin, and produces bleeding from the uterus (Speert, 1940*a*). After estrogenic priming in this species it can also bring about progestational changes in the endometrium and inhibit estrogen-withdrawal

bleeding (Engle, 1941; Zuckerman, 1941). Mammary growth has been produced in male mice by treatment with 0.5 mg. of desoxycorticosterone acetate every other day (Van Heuverswyn, Folley, and Gardner, 1939); and enlargement and tenderness of the breasts were observed in a patient with Addison's disease who received 15 to 20 mg. of this compound daily. When treatment was stopped, these symptoms subsided and the breasts returned to normal (Lawrence, 1943).

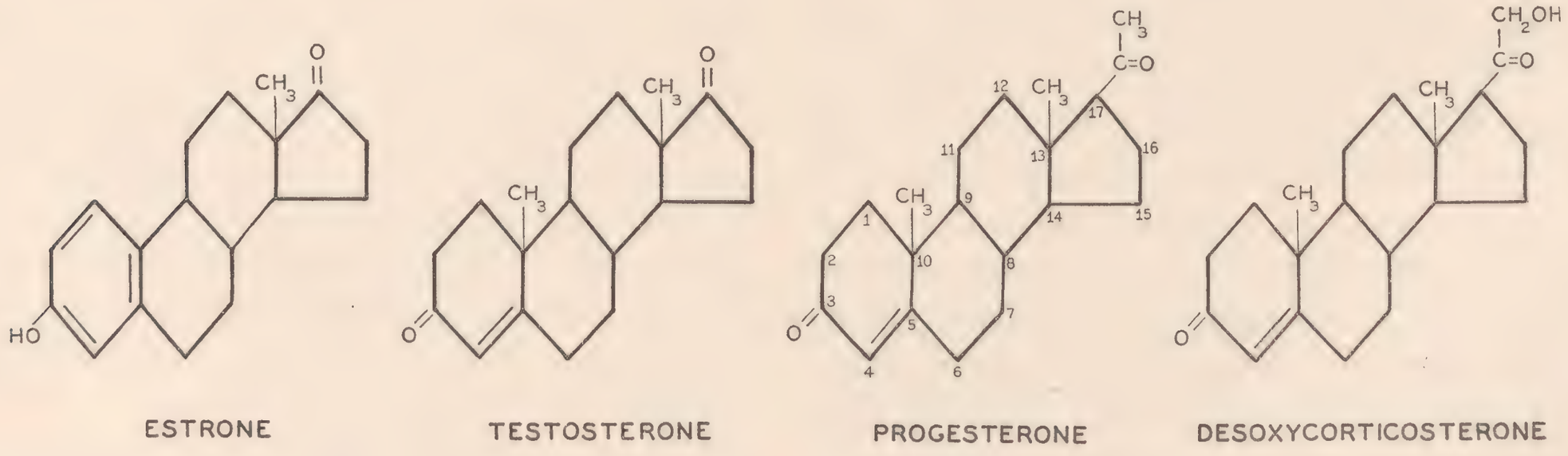
The present experiments include observations on 3 castrated female monkeys which were injected with 5 to 10 mg. of desoxycorticosterone acetate daily for periods up to 30 days. Mammary biopsies were made before and after treatment. Details are shown in table 10. Excellent mammary response was obtained in no. 703 as a result of 24 days' treatment with a 10-mg. daily dose (figs. 107–110, pl. 18). The atrophic lobules enlarged, the alveoli multiplied, their patency was re-established, and numerous mitotic figures appeared in their walls. Proliferation of the duct epithelium also occurred, and a striking increase was found in the thickness of the periductal connective tissue. Little if any mammary development was observed in the biopsy specimen of no. 592 after similar treatment for 13 days. Another biopsy 11 days later, however, revealed distinct glandular growth, though no treatment was given during the interim. Thus, a daily dose of 10 mg. was adequate for mammary stimulation, even over as short a period of treatment as 13 days, but more time was needed for the manifestation of its effect. The latent period required by the monkey's mammary gland for response to hormonal treatment is variable. Monkey no. 620 showed no mammary response to a 5-mg. daily dose of desoxycorticosterone acetate after 30 days' treatment, even though the total amount received during this period was greater than that given no. 592 in 24 days. Obviously, the intensity of hormonal stimulation is more important for mammary development than is the total quantity of test substance injected.

TABLE 10

MONKEYS TREATED WITH DESOXYCORTICOSTERONE ACETATE

| MONKEY | SEX | CASTRATED | WEIGHT (GM.) | TREATMENT | | MAMMARY RESPONSE |
|---|---|---|---|---|---|---|
| | | | | Daily dose | Days | |
| 703.. | F(c) | 6 wks. | 4880 | 10 mg. | 24 | + |
| 592.. | F(c) | 8 wks. | 5070 | 10 mg. | 13 | − |
| | | | | No treatment | 11 | + |
| 620.. | F(c) | 6 wks. | 5520 | 5 mg. | 30 | − |

Desoxycorticosterone is intimately associated with the adrenals. It has been isolated from adrenal cortical tissue (Reichstein and von Euw, 1938). Dorfman and others (1944) have extracted from the urine of castrated male and female monkeys a material which protects adrenalectomized rats. This material could not be recovered from the urine of adrenalectomized monkeys, but was restored in one animal by the injection of desoxycorticosterone acetate. The metabolism of desoxycorticosterone links it with the sex hormones also. The injection of desoxycorticosterone acetate, for example, results in the excretion of pregnanediol, an end product of progesterone (Hoffman, Kazmin, and Browne, 1943). Such similarities in their behavior, and the capacity of estrone, progesterone, testosterone, and desoxycorticosterone to produce almost identical effects on the mammary gland, are not surprising when one considers their chemical structures:



Each contains a steroid nucleus and, with the exception of estrone, the α-β-unsaturated ketone group at $C_3$–$C_4$ is common to all. Desoxycorticosterone can be derived from progesterone by the mere substitution of a hydroxyl group at $C_{21}$. To be sure, even such minor differences in chemical structure result in altered physiologic potency, so that larger amounts of desoxycorticosterone than of progesterone are required to produce certain progesterone-like actions in experimental animals (Robson, 1939; Van Heuverswyn and others, 1939). Still, in adequate dosage each member of an imposing list of steroid compounds has been found capable of stimulating growth of the secondary and accessory sex organs. Clark and Selye (1942, 1943) have stressed the nonspecificity of the steroids by listing 49 of them that can cause proliferation of vaginal epithelium.

Mammary response to hormones, natural or synthetic, would seem, then, to be a function of the potency of the test substance, dose, duration of treatment, and the degree of uptake and utilization of the hormone by the gland. The possible importance of the last factor should not be overlooked, for occasional cases have been reported of girls with infantile genitalia and enormous, growing breasts (Kurzrok and Wilson, 1940).

## MODE OF ACTION OF HORMONES

### Action on the Gland

A theory of mammary development which was proposed in 1931 by Turner and Frank, namely that the mammary ducts develop in response to ovarian estrogens, the lobules of alveoli in response to the added stimulus of progestin, served for about a decade as the basis of mammary developmental physiology. More recent observations, however, have necessitated reconsideration of the mode of action of hormones upon the mammary gland and a modification of the original simple scheme.

The demonstration by Corner (1930), by Asdell, Brooks, Salisbury, and Seidenstein (1936), and by others that mammary growth resulted from the injection of aqueous alkaline pituitary extracts into castrated virgin rabbits, and the finding by Lyons and Catchpole (1933) that growth as well as secretion occurred in the glands of ovariectomized virgin rabbits following the injection of lactogenic hormone, suggested the importance of the pituitary for mammary growth through some mechanism other than its gonadotrophic action. Evidence of the indispensability of the pituitary was furnished by several reports which described the failure of estrogens to stimulate mammary growth in hypophysectomized rats (Selye, Collip, and Thomson, 1935*a;* Reece, Turner, and Hill, 1936; Nelson and Tobin, 1936; Selye and Collip, 1936; Reece and Leonard, 1941). In animals with intact pituitaries, on the other hand, or animals which received pituitary implants or injections of pituitary extracts simultaneously with estrogens, the latter provoked their characteristic extension of the mammary duct system. Similar findings were described in male mice (Gomez, Turner, Gardner, and Hill, 1937). Although no mammary response occurred in completely hypophysectomized mice which were treated with various estrogens, a small fragment of the pituitary, insufficient to maintain the gonads or adrenal cortex, sufficed to allow growth of the mammary glands following the administration of estrogenic hormones. In the guinea pig, too, estrogens induced little if any mammary growth after removal of the hypophysis (Vargas, 1943), although the nipple response appeared to be unaffected by the absence of this gland (Lyons and Pencharz, 1936; Gomez and Turner, 1936). Further experiments on rabbits, cats, and ground squirrels extended to these species the observation that estrogen, even when administered together with progestin, was ineffectual in stimulating mammary growth in hypophysectomized animals. Lacassagne and Chamorro (1939) have also described the failure of estrogenic hormones to induce mammary cancer in hypophysectomized mice of a susceptible strain.

Other steroid hormones of known mammotrophic activity such as testosterone (McEuen, Selye, and Collip, 1937), testosterone propionate (Noble, 1939; Leonard and Reece, 1942; Reece and Leonard, 1942), and desoxycorticosterone acetate (Chamorro, 1940*a*), either alone or in combination with estrogen, likewise have been found to exert no effect on the mammary glands of hypophysectomized rats and mice, although growth of the nipples and retardation of the regression of the secondary sexual organs were observed.

Evidence has been presented that mammary de-

velopment can be produced in hypophysectomized male guinea pigs by the implantation of pituitaries from animals of the same or other species which have been treated previously with estrogen, whereas the hypophyses of untreated donors are relatively ineffectual (Gomez, Turner, and Reece, 1937; Gomez and Turner, 1937). Reece and Leonard (1939) and Gomez (1942) later found that the pituitaries of untreated as well as estrogen-treated rats of both sexes exerted a mammotrophic effect on castrated and on sexually immature females.

Nelson (1939) was unable to demonstrate an increase in the mammotrophic activity of the rat pituitary after pretreatment with estrone, although Gomez and Turner (1938*b*) had observed such an augmentation in the rabbit after injection of anol as well as steroidal estrogens. The latter authors concluded that estrogens produce their effect upon the mammary gland through the mediation of the pituitary.

The anterior pituitaries of pregnant cattle were found to be a good source of this pituitary "mammogen"; the glands of nonpregnant cattle, on the other hand, despite their content of lactogenic hormone, contained little if any "mammogen," according to Gomez and Turner (1938*a*). These investigators also postulated the existence of two distinct "mammogenic factors" in the anterior pituitary: a "duct growth factor," formed as a result of estrogenic stimulation, and a "lobule-alveolar growth factor," a product of progestogenic activity.

The duct growth factor reached its highest concentration in the cow's pituitary at about the 150th day of pregnancy and differed from other hormones of the anterior pituitary in being soluble in lipid solvents and in not being isoelectrically precipitated from alcohol. It was distinguished from estrogen by being heat labile and subject to oxidation (Lewis and Turner, 1939). An assay method was developed, based on the mammary development which occurs in immature male mice after the daily injection of this factor for 6 days (Lewis, Turner, and Gomez, 1939). Mammary gland development with the duct growth factor, also known as "mammogen I," was later described in the castrate and in the hypophysectomized rat (Lewis, Gomez, and Turner, 1942).

Evidence has also been adduced for the existence of the second "mammogenic factor" in the anterior pituitary ("mammogen II"; Mixner, Lewis, and Turner, 1940), and a method for its assay on ovariectomized virgin mice has been described (Mixner and Turner, 1941). Recent analyses have shown this factor to be protein in nature, in common with other hormones of the anterior pituitary, but probably distinct from the lactogenic, thyrotrophic, and gonadotrophic hormones (Mixner, Bergman, and Turner, 1942).

Before these "mammogenic factors" can be accepted as new and distinct entities, several legitimate objections must be answered. Despite the negative results of one assay for estrogen, extracts of the pituitaries, especially of pregnant or estrogen-treated animals, might have contained small amounts of estrogenic hormone which, either alone or in combination with other pituitary factors, could induce a mammary response of sufficient degree to be considered positive by the "mammogen" assay technique of Lewis, Turner, and Gomez.[5] Since extensive development of the nipples as well as the glands occurred in the hypophysectomized male guinea pigs in which Gomez, Turner, and Reece (1937) implanted pituitaries of estrogen-treated donors, it seems likely that more than negligible amounts of estrogen were transferred with the implants. Employing the McGinty intra-uterine technique, Trentin, Mixner, Lewis, and Turner (1941) obtained negative assays for progesterone with amounts of fresh pregnant cattle pituitary which were capable of stimulating growth of mammary lobules in castrated female mice; negative assays for progesterone were likewise obtained with lipid pituitary extracts which stimulated duct growth in the male mouse. The uterine response to progesterone might have been inhibited, however, by a sufficient concentration of estrogen in the test material. Also, stimulation of the adrenals might have occurred if any adrenotrophic hormone was present. Several steroids of adrenal origin, including estrogens, androgens, progesterone, and desoxycorticosterone, have mammotrophic activity. Nelson (1941*b*) has induced growth of the mammary glands of gonadectomized-hypophysec-

[5] In a more recent report Trentin, Lewis, Bergman, and Turner (1943) have admitted the possibility of estrogen contamination in the lipid pituitary extracts. Using improved extraction procedures these authors now believe "mammogen I" to be protein rather than lipid in nature. In another paper Mixner and Turner (1943) reported that pituitary "mammogenic" preparations were relatively inert when injected into ovariectomized virgin mice unless estrogen was administered simultaneously.

tomized immature mice of both sexes by the administration of adrenocorticotrophic hormone, whereas mammary growth failed to occur following such treatment of gonadectomized-adrenalectomized animals. Further, if gonadotrophins were present in the pituitary extracts, testicular androgens could have been produced, since intact rather than castrated male mice were used in the assay method of "mammogen I." These objections could be met, at least in part, by the use of adrenalectomized castrated animals for assay purposes. Unqualified acceptance of the concept of specific "mammogenic" hormones of pituitary origin appears, therefore, not to be justified at the present writing.

On the contrary, evidence from other sources has furnished a sound basis for questioning such a concept. First, during pregnancy the placenta is capable of sustaining the growth of the mammary glands. It has been amply demonstrated that mammary development can continue in the absence of the pituitary in the pregnant rat (Pencharz and Long, 1933; Jeffers, 1935), mouse (Newton and Richardson, 1941; Gardner and Allen, 1942), and guinea pig (Desclin, 1939). Also, gonadotrophic hormone from pregnancy urine or pregnant mare serum can maintain the mammary glands of hypophysectomized mice with intact ovaries (Chamorro, 1940*b*). When the uterus of the mouse was emptied at mid-pregnancy, mammary involution promptly followed (Newton and Beck, 1939).

Second, positive experimental evidence contradicts some of the afore-mentioned negative findings and indicates that the sex hormones can stimulate mammary growth in hypophysectomized animals. In hypophysectomized rats, for example, glandular development has been produced by injections of estrogen and desoxycorticosterone acetate in combination (Chamorro, 1940*c*), estrogen and progesterone, either singly or together (Smithcors and Leonard, 1943), estradiol dipropionate, testosterone (Leonard, 1943), and a combination of estrone and purified lactogenic hormone (Lyons, Simpson, and Evans, 1942). Similar results have been obtained in hypophysectomized mice with estradiol dipropionate, desoxycorticosterone acetate, and progesterone, singly or in various combinations with each other (Gardner, 1940, 1941*a*) and with prolactin (Gardner and White, 1942*a*, 1942*b*); in hypophysectomized rabbits treated with estrogen, either alone or in combination with progestin (Asdell and Seidenstein, 1935); and in hypophysectomized guinea pigs treated with estrone (Nelson, 1935).

The failure of mammary development to occur in some hypophysectomized animals which were treated with estrogens has been attributed to the nonspecific effects of inanition resulting from removal of the pituitary (Astwood, Geschickter, and Rausch, 1937). Subsequent experimental work has given support to this explanation. When "growth hormone" plus estrogen was administered to hypophysectomized rats in which estrogen alone had failed to elicit mammary growth, distinct stimulation of the glands was observed (Reece and Leonard, 1941). Similar glandular responses resulted from testosterone propionate when growth hormone was also injected into the hypophysectomized rats (Reece and Leonard, 1942). Samuels, Reinecke, and Petersen (1941) succeeded in maintaining the body weight of hypophysectomized rats by forced feeding. Yet the mammary glands of these animals failed to respond to estradiol benzoate. The existence of complicating metabolic disturbances is suggested, however, by the failure of growth in the nipples also. In another series of hypophysectomized rats which were treated with estradiol benzoate and growth hormone, the role of nutrition was stressed by the correlation between the weight loss of the animal and the degree of atrophy of its mammary glands (Nathanson, Shaw, and Franseen, 1939). Similarly, a general, although not rigid, correlation was found between the extent of mammary stimulation and the amount of body growth that occurred in hypophysectomized rats treated with desiccated cattle pituitary, whereas lipoid pituitary extracts (which should have contained "mammogen") were without effect on the mammary glands (Greep and Stavely, 1941). Moreover, in intact albino mice on a limited food intake, the amount of estrogen required to elicit a standard growth response in the mammary glands increased in proportion to the degree of dietary restriction (Trentin and Turner, 1941).

Finally, critical evidence resulting from a new method of approach has been brought to bear against the claims for specific mammotrophins of pituitary origin. Unilateral growth of the mammary glands has been produced by the direct topical application of estrogen to the nipple area. This type of experiment

has permitted the use of normal intact animals, and the complicating factors of surgical interference and nutritional disturbances have thereby been obviated (Speert, 1940*b*).

Three immature male monkeys were used, their average weight being about 2400 gm. To the left nipple of each animal a small amount of estrone was applied daily. This was accomplished by "painting" the nipple with an alcoholic solution of estrone containing 0.05 mg. of the estrogen per cc. of 95 per cent ethyl alcohol. Preliminary experiments with guinea pigs had shown that the most pronounced differential growth between the two nipples could be obtained with this concentration of the hormone. When weaker solutions were used, the rate of growth of the nipple was slower; when higher concentrations of estrone were applied, systemic absorption occurred and both nipples responded. The right nipple of each monkey was painted with plain alcohol and served as a control. Two animals were so treated for 75 days; the entire mammary glands were then removed surgically. The third monkey was treated for only 50 days, but 105 days were then allowed to elapse before the mammae were removed. Histologic sections were made of the mammary tissue at the base of the nipple; the rest of the gland was prepared as a gross mount.

In each case the estrogen-treated gland was distinctly larger and better developed than the control. The spreads of the former had a greater area, their ducts were wider and had more numerous branches, and clusters of end buds were present on many of their terminal arborizations. The resultant mammary asymmetry was greatest in the monkeys which were treated for 75 days (figs. 111, 112, pl. 18), but a difference between the two breasts was still discernible in the third animal, which was treated for a shorter period and whose mammary glands were not examined until 3½ months later. Even in this case the area of the left mammary gland was about 50 per cent greater than that of the right.

An equally striking contrast was afforded by the histologic sections of the estrogen-treated and the control glands. In a typical section of the latter, only a single small duct could be seen, lying in a loose connective-tissue stroma (fig. 113, pl. 18), whereas sections taken from a comparable region of the estrogen-treated breast of the same animal contained several extremely hyperplastic ducts which were surrounded by a dense mantle of connective tissue (fig. 114, pl. 18).

The local response of the mammary gland to the cutaneous application of estrogen has been confirmed in the monkey (Chamberlin, Gardner, and Allen, 1941) and has been demonstrated similarly in the rabbit (Lyons and Sako, 1940; Lewis and Turner, 1942), mouse (Gardner and Chamberlin, 1941), guinea pig (Nelson, 1941*a*), and cow (Petersen, personal communication, 1943). There is also some evidence that unilateral mammary development can be produced in the human by local application of estrogens (MacBryde, 1939), whereas no effect is produced by several fraudulent products which have appeared on the commercial market from time to time, advertised as "bust developers" (Jour. Amer. Med. Assoc., 1940, 1943, 1944).

These experiments leave little room for doubt as to the mode of action of estrogens upon the mammary gland. Under simple experimental conditions which have minimized opportunities for discrepancies in technique and disagreement on interpretations, several workers in different laboratories have obtained similar results in a variety of species. From these observations one can only conclude that estrogens produce their trophic effect upon the mammary gland by direct action. Therefore, no support can be offered for the pituitary mediation of this effect upon the mammae. Such a mode of action would be expected to cause a similar growth response in both mammary glands rather than the unilateral effect which was produced by locally applied estrogen. Though these observations fail to support the theory of pituitary mammotrophins, they still do not preclude their possible existence. Estrogens have merely been proved capable of acting directly upon the mammary gland. Other channels may also exist through which estrogenic action on the breast is effected, but these must await further experimental demonstration.

A recent report by Lyons (1942) has indicated that the lactogenic hormone also can act locally upon the mammary gland. When this substance was injected intraductally, through the nipples of rabbits, localized lactation ensued in the injected parts of the glands while the control sectors remained unaffected.

## Action on the Nipple

Although the manner in which hormones produce their effects upon the mammary gland has been the subject of considerable controversy, general agreement prevails concerning the mode of action of estrogens upon the nipple. No claims have appeared for other than a direct effect, even though growth of the nipples was not observed in some hypophysectomized animals which had been injected with estrogens (Samuels, Reinecke, and Petersen, 1941). In the vast majority of experiments in which adequate amounts of estrogenic hormone were injected into rats, rabbits, mice, and guinea pigs, a rather prompt nipple response was observed in hypophysectomized as well as in intact animals. Likewise when estrogen was applied by inunction to the skin of hypophysectomized male rats, growth of the nipples resulted (Ruinen, 1932).

By topical application of estrogenic hormone to the nipple areas of various species it has been possible to elicit localized growth responses in the treated nipples which have been even more striking than the previously mentioned local effects of the hormone upon the mammary glands proper. This finding was first described in the guinea pig by Zondek in 1935. Percutaneous application of estrogen resulted in enlargement of the treated nipple in 8 days. On continued treatment, however, sufficient systemic absorption occurred so that after 4 weeks no difference between the two nipples was discernible. Subsequent experimenters have obtained a unilateral nipple response in the guinea pig by applying various estrogens to the nipple area in a variety of oils, ointment bases, and alcohol (Mussio Fournier, Albrieux, and Buño, 1937; Jadassohn, Uehlinger, and Zürcher, 1937; Jadassohn, Uehlinger, and Margot, 1938; Fierz, 1939; Jadassohn, Uehlinger, and Fierz, 1941; Nelson, 1941*a*). Jadassohn, Uehlinger, and Margot have also obtained localized growth of the guinea pig nipple by the topical application of aqueous solutions of testosterone propionate, androsterone, androstenedione, androstanedione, andrenosterone, and corticosterone. Similar but less striking has been the nipple response of the rabbit to locally applied estrogens (Lyons and Sako, 1940; Lewis and Turner, 1942).

During the course of the previously described experiments in which the left nipple of immature male monkeys was painted daily with an alcoholic solution of estrone, frequent measurements were made of both the estrogen-treated and the control (alcohol-treated) nipples. These measurements included the length of the nipple, the diameter of its base, and the diameter of the areola. For purposes of comparison, the nipple was considered to be a cone, and its volume was calculated accordingly by the formula $V = \frac{1}{3}\pi r^2 h$, where $V$ represents the volume of the nipple, $r$ the radius of its base, and $h$ its length. In a similar fashion, considering the areola to be a circle, its area was calculated by the formula $A = \pi r^2$, where $A$ represents the area of the areola, and $r$ its radius. In the first group of two animals which were so studied (L1 and L2), the contrast between the estrogen-treated nipples and areolas on the one hand, and the control nipples and areolas on the other was so striking (fig. 1) that it was considered desirable to study this phenomenon of differential growth under a variety of conditions in additional animals.

In all, 18 monkeys were studied. The left nipple was painted daily with an alcoholic solution of estrone (0.05 or 0.5 mg. per cc.); the right nipple, serving as a control, was painted with plain alcohol. In two cases (L1 and 708) in which supernumerary nipples were present, these also were painted with the estrogenic solution. In one animal (G25), the right nipple had been amputated prior to this experiment. The measurements of the nipples and areolas at the beginning and at the end of treatment are recorded in table 11. In 4 experiments (S3, S6, S2, and S10), in which the observations were continued for a period beyond the cessation of treatment, the last of these measurements is included in the table.

The experiments were divided into five main groups:

1. *Group I* (monkeys L1 and L2). The left nipples of these two immature males were treated for 75 days with a solution of estrone containing 0.05 mg. per cc. The supernumerary nipple of one of the animals (L1) was treated similarly with the hormone. The volume of the estrogen-treated and the control nipples and the area of the experimental and control areolas are plotted in figure 1. The curves are based on the average measurements of both animals at each stage of treatment. The left nipple underwent a 14-fold increase in volume during the 75 days of treatment,[6]

[6] The supernumerary nipple showed a proportionately greater increase in volume (36-fold).

while the volume of the right nipple remained relatively unchanged. The areola of the left nipple increased in area in a similar although less striking fashion, in contrast with the insignificant changes in the area of the right areola.

2. *Group II* (monkeys S3, S6, S2, and S10). These immature males were treated for 50 days. Treatment was then stopped, but measurements were made periodically during the next 85 days. The animals were divided into two subgroups:

*Group II-A* (monkeys S3 and S6) was treated with a solution of estrone containing 0.05 mg. per cc. During the 50 days of treatment, the average volume of the left nipples, as in group I, underwent a sharp

TABLE 11

EFFECT OF TOPICAL APPLICATION OF ESTROGEN ON NIPPLE

(Left nipple and supernumerary treated with estrone; right nipple, control)

| MONKEY | DAY | RIGHT NIPPLE (MM.) | | | LEFT NIPPLE (MM.) | | | SUPERNUMERARY (MM.) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Length | Width | Areola | Length | Width | Areola | Length | Width | Areola |
| L1 | 1 | 1.5 | 2.5 | 7.5 | 1 | 2.5 | 7 | 0.5 | 2 | 5 |
| | 75 | 2 | 3 | 7 | 4.2 | 5 | 11 | 3 | 5 | 6 |
| L2 | 1 | 1.5 | 2.5 | 7 | 1.3 | 2.5 | 6 | | | |
| | 75 | 1.5 | 3 | 7.5 | 4.3 | 5 | 9.5 | | | |
| S3 | 1 | 2.5 | 2.3 | 7 | 3 | 2.5 | 7 | | | |
| | 50 | 4 | 2.5 | 8.5 | 6.5 | 6 | 13 | | | |
| | 135 | 3 | 2.5 | 9 | 4 | 4 | 12 | | | |
| S6 | 1 | 2.5 | 2.3 | 6 | 2.5 | 2.3 | 6 | | | |
| | 50 | 4 | 4 | 8 | 8.5 | 6 | 15 | | | |
| | 135 | 3.3 | 4 | 8.5 | 5 | 5 | 12 | | | |
| S2 | 1 | 3 | 2.3 | 6 | 2.5 | 2.3 | 6 | | | |
| | 50 | 4 | 2 | 7 | 6 | 5 | 14.5 | | | |
| | 135 | 3.5 | 2 | 7.5 | 3.5 | 4 | 9.5 | | | |
| S10 | 1 | 2.5 | 2 | 6 | 2.5 | 2 | 6 | | | |
| | 50 | 4 | 2.5 | 8 | 5.5 | 5.5 | 14 | | | |
| | 135 | 3.5 | 2 | 9 | 3.3 | 3.5 | 10 | | | |
| 708 | 1 | 8 | 6.5 | 12.5 | 9 | 7 | 14 | 5 | 6.5 | 9 |
| | 33 | 9 | 6.5 | 9 | 9 | 7 | 12.5 | 5.5 | 5 | 7.5 |
| 628 | 1 | 8.5 | 6.5 | 15 | 8.5 | 6 | 15 | | | |
| | 61 | 9.5 | 7.3 | 16 | 9.5 | 7.3 | 16 | | | |
| 636 | 1 | 9 | 5.5 | 12 | 14 | 6 | 15 | | | |
| | 61 | 9.8 | 5.3 | 8 | 15 | 6.3 | 15 | | | |
| 651 | 1 | 10.5 | 7.5 | 22 | 10 | 7 | 20 | | | |
| | 61 | 10 | 7.5 | 19 | 9.8 | 8 | 20 | | | |
| G24 | 1 | 5.2 | 4 | 16 | 5 | 4 | 14 | | | |
| | 50 | 5.3 | 4.3 | 16 | 5.5 | 4 | 15 | | | |
| G25 | 1 | .... | .... | .... | 2.5 | 3.5 | 9 | | | |
| | 50 | .... | .... | .... | 4 | 3 | 11 | | | |
| G28 | 1 | 3.2 | 3 | 9 | 3 | 3.5 | 8.5 | | | |
| | 50 | 3.2 | 3.5 | 8.5 | 3 | 4 | 10 | | | |
| G29 | 1 | 1.5 | 4 | 9 | 2 | 4 | 9 | | | |
| | 50 | 1.8 | 3 | 12 | 2 | 3 | 12 | | | |
| G32 | 1 | 3.5 | 3.3 | 10 | 3 | 3 | 13 | | | |
| | 50 | 3.5 | 3.5 | 12.5 | 3.5 | 3.3 | 13 | | | |
| G33 | 1 | 4 | 4 | 12.5 | 2 | 4.5 | 9 | | | |
| | 50 | 4.5 | 4.5 | 11 | 2 | 4 | 7 | | | |
| G35 | 1 | 6.3 | 4.5 | 10.5 | 6.2 | 5.5 | 13 | | | |
| | 50 | 6.5 | 5 | 7.5 | 5 | 5 | 7 | | | |
| G37 | 1 | 6.3 | 5 | 13 | 5.5 | 4.5 | 13 | | | |
| | 50 | 6.5 | 5 | 11 | 6 | 5 | 10 | | | |

14-fold increase. The control nipples also increased in volume during this period, but to a relatively insignificant degree (fig. 2). When treatment was stopped, the left nipples involuted rapidly during the following month. After this initial decline, however, the volume curve leveled off, so that at the end of 85 days the average volume of the left nipples was still 2½ times as great as the average volume of the right nipples and 5 times as great as the average volume of the left nipples before treatment was begun.

The average area of the left areolas at the end of the 50-day period of estrogenic treatment was more than 5 times the average of the pretreatment areas (fig. 3). During the same period the right areolas grew only slightly. Following the cessation of treatment the estrogen-treated areolas shrank to one-half their former area within a month; but they maintained this size, which was still significantly in excess of the average for the right areolas, to the end of the period of observation.

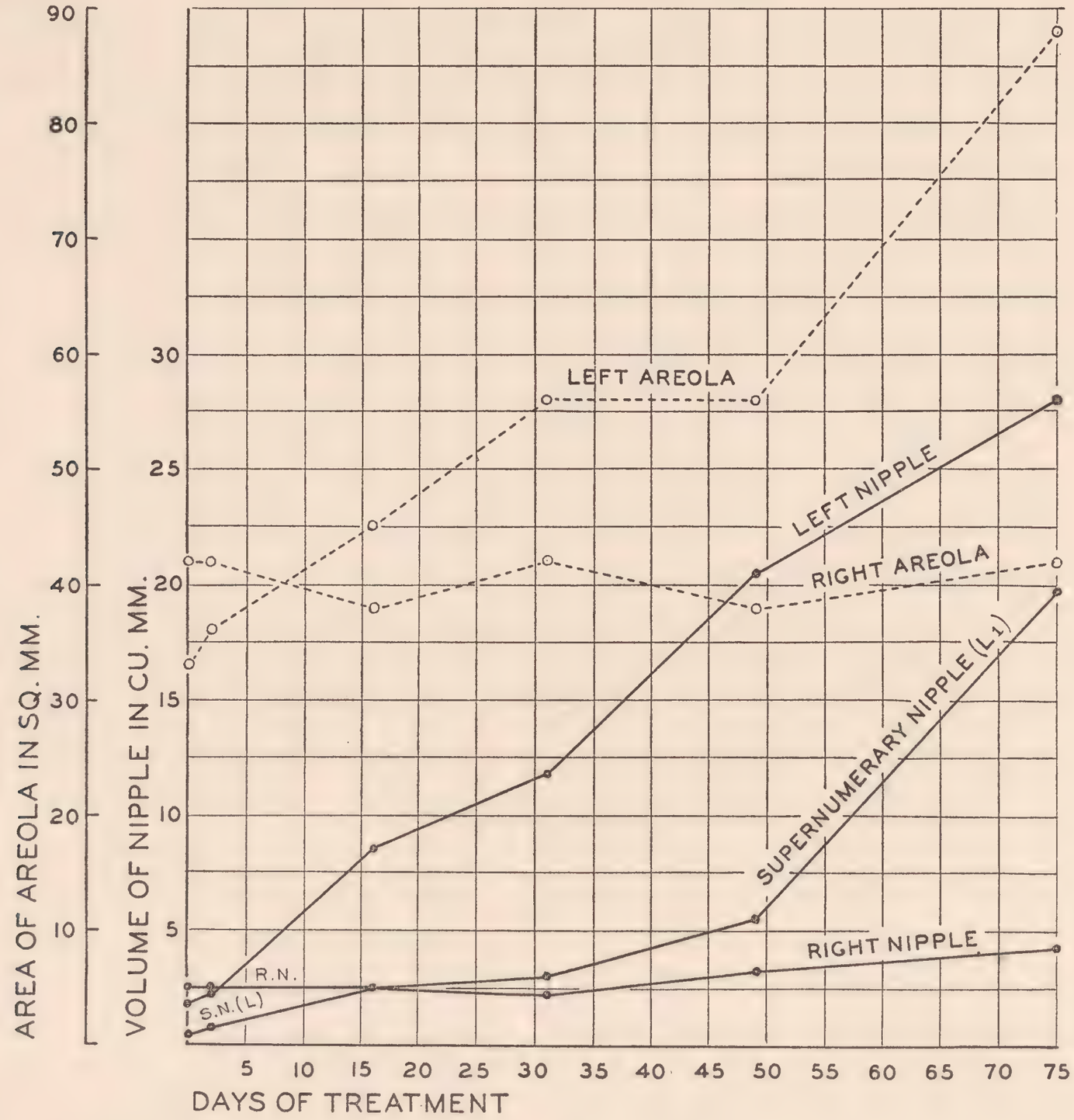



FIG. 1. The effect of locally applied estrone on the volume of the nipple and the area of the areola

*Group II-B* (monkeys S2 and S10) was treated with a solution of estrone containing 0.5 mg. per cc. The nipple and areolar responses in these animals were similar qualitatively to those in group II-A (figs. 2, 3).

3. *Group III.* This group consisted of a single animal, no. 708, a mature female with a supernumerary nipple on the right side. Estrone (0.05 mg. per cc.) was applied to the left nipple and to the supernumerary nipple for 33 days, with no significant increase in the size of either nipple or areola resulting (table 11).

4. *Group IV* (monkeys 628, 636, and 651). These 3 animals were at various stages of pregnancy while receiving treatment with a solution of estrone (0.05 mg. per cc.) for 61 days. The nipples and areolas were unaffected (table 11).

5. *Group V* (monkeys G24, G25, G28, G29, G32, G33, G35, and G37). This group of young animals consisted of 7 females and 1 male. All had been

receiving intensive estrogenic treatment, in the form of subcutaneous pellets and oily injections, for periods ranging up to 2 years. The topically applied estrone was without demonstrable effect on either the nipples or the areolas after 50 days' treatment (table 11).

The response of the nipple and areola to the topical application of estrone was limited to the immature males in groups I and II. The mature female and the 3 pregnant animals which received the same treatment were unaffected. Similarly, the nipples and areolas of the immature animals which were undergoing treatment with estrogen pellets and injections failed to respond to the topically applied hormone. It is not surprising that the nipples of the pregnant monkeys and those of the immature animals in group V did not increase in size following the percutaneous application of estrogen, for these animals were already "saturated" with estrogens. Therefore no significant

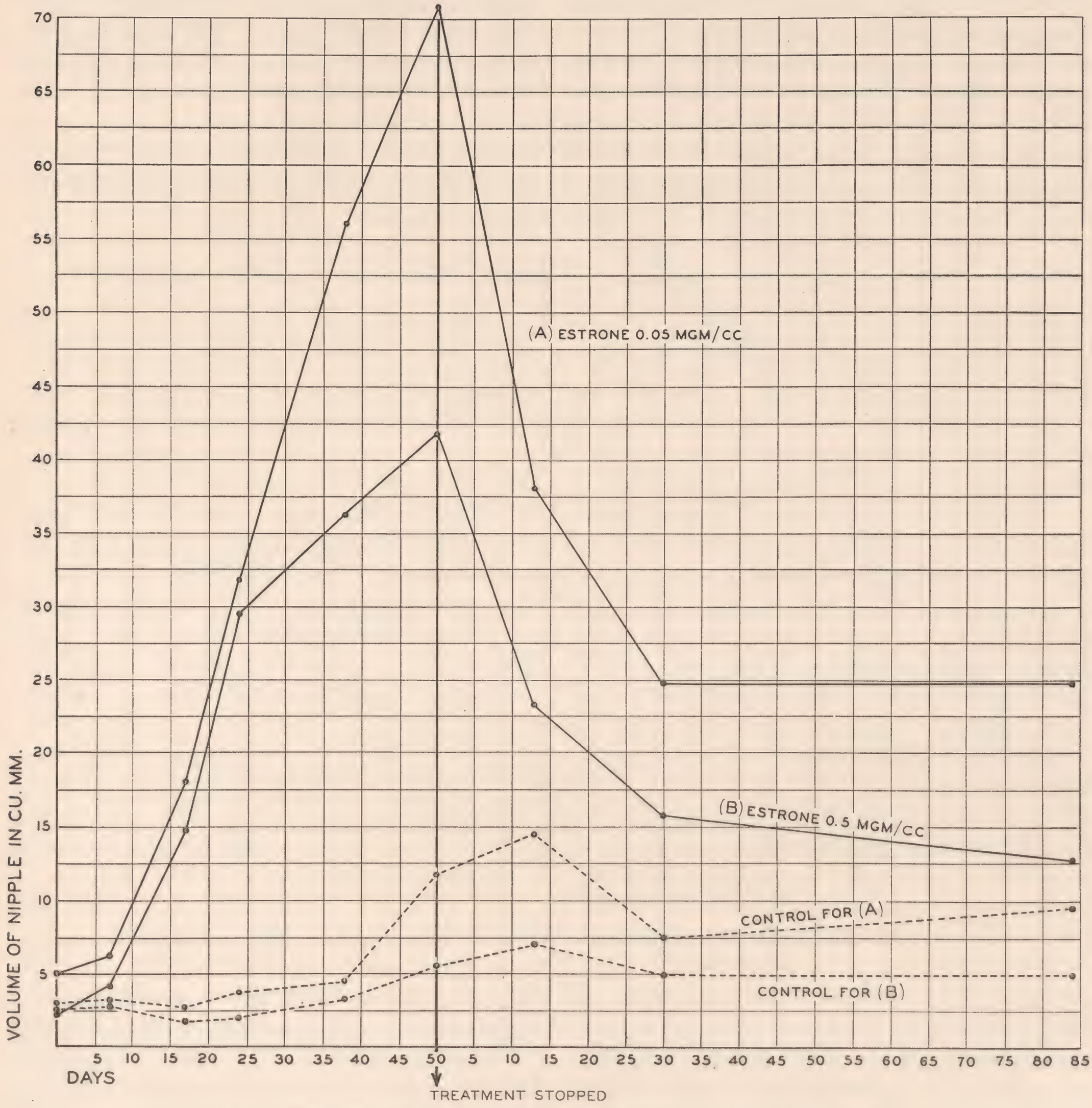



FIG. 2. The effect of locally applied estrone on the volume of the nipple

increase in the already high titer of the hormone in the tissues was to be expected. As a result of the intense estrogenic stimulation to which they had been subjected, the nipple structures had already realized their maximal possibilities for growth.

Chamberlin, Gardner, and Allen (1941) have observed a similar, although less striking, differential growth in the nipples of 2 young male monkeys following treatment of the left nipple with an alcoholic solution of estrone. A slight response was observed

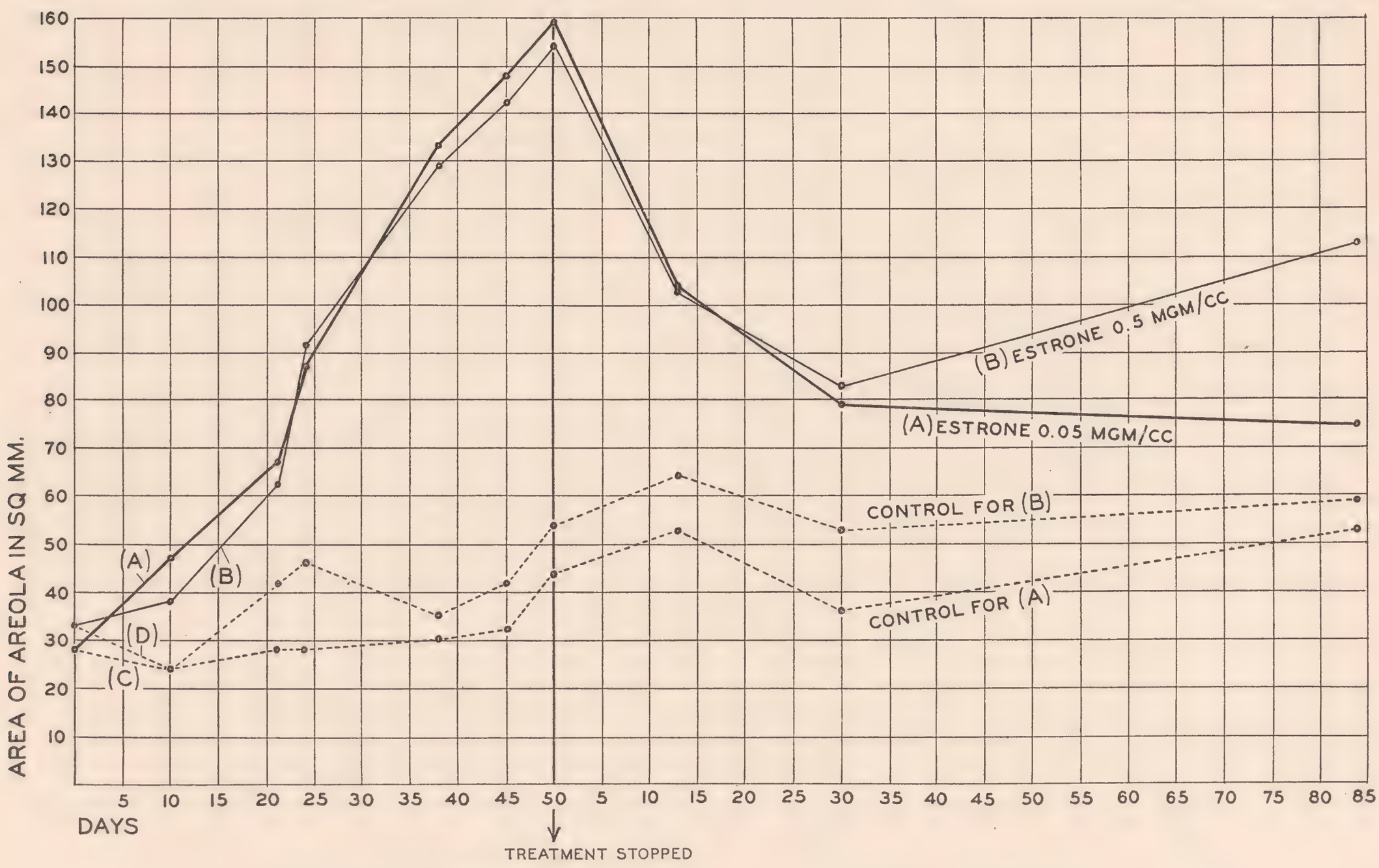



FIG. 3. The effect of locally applied estrone on the area of the areola

in the nipples of 3 out of 6 human newborns who were treated in this manner (Speert, unpublished).

Significant histologic differences between the estrogen-treated and the control nipples were found in the monkeys in groups I and II. These differences occurred most consistently in the subepithelial tissues. Outstanding was the increase in the number and dilatation of the lymphatic vessels in the estrogen-treated nipples. The connective-tissue stroma of the experimental nipples increased in quantity and in cellularity and appeared to contain a greater quantity of interstitial fluid. In some cases hypertrophy of the smooth muscle bundles occurred in the estrogen-treated nipple; this was particularly striking in monkey no. L1. The vascularity of the experimental nipples underwent an increase which varied in degree among the different animals. The blood capillaries were conspicuously filled with red blood cells. This accounted for the pinkish coloration which the nipples and areolas assumed during treatment (figs. 115, 116, pl. 19). Vascular dilatation was also observed in the experimental nipples of several of the mature animals (especially the supernumerary nipple of no. 708), despite the failure of these nipples to increase in size.

In each case except one, the epithelial coverings of the two nipples were indistinguishable. In monkey no. L2, however, a significant difference was observed between the nipple epithelium of the experimental and that of the control side. Mitotic figures were common in the Malpighian layer of the estrogen-treated nipple, and the stratum germinativum had increased to several times the thickness of the corresponding layer in the control nipple (figs. 117, 118, pl. 19). The absence of similar epithelial hyperplasia in the nipples

of the other animals is unexplained. This reaction occurred uniformly in guinea pigs whose nipples were treated by topical application of estrogens (Fierz, 1939; Jadassohn, Uehlinger, and Fierz, 1941). The increased pigmentation which was observed in the nipples of estrogen-treated guinea pigs (Davis, Boynton, Ferguson, and Rothman, 1945) was also absent in our monkeys. The stromal reaction which occurred in the absence of significant epithelial hyperplasia is indicative of the sensitivity of the lymphatics and capillaries of the nipple to estrogenic stimulation. Estrogens can also produce a specific increase in the permeability of the capillaries of the rat uterus and vagina (Hechter, Krohn, and Harris, 1942).

## SUMMARY

1. The mammary line was demonstrated in a monkey embryo of 34 days (crown-rump length 11.3 mm.).

2. The mammary gland of the newborn consisted of a few short, poorly branched ducts lined with hyperplastic, nonsecretory epithelium.

3. Extension and branching of the mammary ducts occurred during adolescence, with a resultant increase in mammary area. The right and left breasts grew at about the same rate.

4. The adult breast consisted of a flat sheet of mammary tissue subjacent to the skin, extending laterally into the axilla and caudally below the costal margin.

5. Pronounced individual variation was found in the mammary glands of both male and female monkeys under identical physiologic and experimental conditions. Changes in the gland were therefore studied by means of repeated biopsies in the same animal.

6. Supernumerary nipples were discovered in 14 monkeys of the Carnegie collection, an incidence of about 1.4 per cent. In 6 cases mammary ducts were associated with the extra nipple.

7. Lobules of alveoli were present in only a few of the male breasts.

8. Cyclic changes occurred in the mammary glands of mature females, in relation to the menstrual cycle. Lobular enlargement, resulting from dilatation of the alveoli, was observed during the premenstruum and gradually subsided during the postmenstrual period. This change occurred in association with ovulatory cycles; no mammary changes were observed when ovulation failed to occur.

9. Removal of the corpus luteum 5 days after ovulation resulted in a rapid shrinkage of the mammary lobules.

10. Hysterectomy produced no effect on the mammary gland.

11. During the first 2 months of pregnancy a high degree of individual variation was found in the mammary gland, little growth occurring during this period. The 3d month witnessed a sharp increase in the rate of glandular development. Secretory activity was usually well established microscopically by the end of the 4th month of pregnancy. Mitotic proliferation of the gland cells continued to term. At term the mammary gland was uniformly developed; no "virginal lobules" remained.

12. Removal of both ovaries as early as the 31st day of pregnancy had no effect on the breast; glandular development and lactation proceeded normally.

13. Secretory changes occurred in the gland within 2 days after the termination of pregnancy at 60 days.

14. Lactation began within 2 days after parturition. When lactation was at its height and the breasts hung in large folds below the costal margins, secretory activity was present in the duct epithelium. Mitotic figures became rare after the first few days of the puerperium, and during advanced lactation they could not be demonstrated at all. Many lactating cells contained double or lobulated nuclei.

15. Postpartum and postlactational involution of the mammary gland occurred irregularly, progressing at different rates in different lobules, in contrast with the uniform development of the gland during pregnancy. On the average, the lobules remained engorged for about 2 weeks after parturition or after the cessation of lactation. Involutionary processes became evident during the 2d week and were complete at the end of 3½ months. Reduction of glandular tissue resulted in part from atrophy of the cells, and in part from desquamation of the cells into the alveoli and ducts. Involution appeared to progress more rapidly in animals which had been permitted to nurse their young than in those whose young were taken from them at birth.

16. After fetal death in utero, mammary involution began irrespective of expulsion of the fetus. Involu-

tionary processes were observed following the interruption of pregnancy as early as the 36th day.

17. An unusual, irregular mammary pattern was observed in a pregnant monkey at term, 26 days after weaning her last offspring.

18. Castration of mature female monkeys resulted in generalized atrophy of the mammary gland and the simultaneous development of discrete hyperplastic nodules. Natural or synthetic estrogens, progesterone, androgens, and desoxycorticosterone acetate were each effective in preventing nodule formation when they were administered in adequate dosage within the first month after castration.

19. Stimulation of mammary growth was produced by estrogens, progestogens, androgens, gonadotrophins, and desoxycorticosterone acetate. Each hormone caused the same general pattern of response, which consisted of hyperplasia, dilatation, extension, and branching of the ducts, and growth of the lobules by multiplication and dilatation of the alveoli. Growth was produced in castrated mature females by 100 r.u. of an estrogen daily for 50 days. When the ovaries were present, stimulation occurred in a month with only 50 r.u. daily. Larger amounts of estrogen resulted in an increase in the speed and the extent of mammary growth. Complete glandular development was produced and maintained in castrated females for periods up to 30 months by continuous treatment with estrogenic injections and subcutaneous implantations of estrogen pellets. The early effects of intense estrogenic stimulation consisted in stratification of the duct epithelium, followed by lobular growth and cystic dilatation of the ducts. As lobular growth continued, the ducts reverted to normal. With 5 mg. of estradiol daily, complete glandular development was produced in prepubertal animals in 70 to 85 days. Stilbestrol, in increasing amounts up to 2 mg. per day for a month, produced no effect.

20. Benign metaplasia of the mammary epithelium occurred in one-third of a group of highly estrogenized monkeys. Small islets of atypical pale epithelium, resembling the type frequently seen in mammary cysts in the human, appeared among the normal lobules and in the duct linings. Prolonged and intense estrogenic treatment failed to produce malignant change.

21. Mammary development was produced in castrated females by the sequential action of an estrogen (Amniotin) and a progestogen (progestin or anhydrohydroxy-progesterone). Crystalline progesterone, in daily doses from 5 to 20 mg., caused mammary growth in castrated females without estrogenic priming. The compact, dense connective tissue of the mammary stroma of castrated animals became loose, fibrillar, and edematous as a result of treatment with progesterone.

22. Gonadotrophins (FSH, Follutein, and Gonadogen) resulted in slight to moderate mammary development in 2 amenorrheic females. Treatment of a third animal with FSH and Follutein had no effect on the breast. Mammary response to gonadotrophins could be correlated with other evidence of ovarian stimulation.

23. Testosterone and testosterone propionate, in amounts ranging from 5 to 50 mg. per day, each produced mammary growth in castrated and intact females. Mammary atrophy occurred when treatment was discontinued. Androstenediol and methyl testosterone (by mouth) were each without effect. Positive mammary responses occurred when testosterone or methyl testosterone was administered concurrently with an estrogen. When estrogen and androgen were administered sequentially, positive responses were obtained with Amniotin together with testosterone, testosterone propionate, or testosterone dipropionate, but not with methyl testosterone (by mouth). Epithelial proliferation which resulted in heaping of the cells and the formation of microscopic papillomas in the ducts and alveoli occurred in 2 females which received large doses of testosterone and testosterone propionate respectively.

24. Desoxycorticosterone acetate, in a daily dose of 10 mg., produced mammary development in castrated females; but a daily dose of 5 mg. had no effect on the breast.

25. Topical application of an alcoholic solution of estrone to one nipple area of prepubertal male monkeys resulted in localized, unilateral growth of the nipple and areola and the underlying mammary tissue; whereas the control gland, treated with alcohol, was unaffected. These observations offer no support for the theory of pituitary mediation of estrogenic effects upon the mammary gland. Similar treatment caused no nipple growth in pregnant animals or in those injected with large amounts of estrogen.

## Acknowledgments

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## Plate 1

Fig. 4. Embryo C 665, crown-rump length 11.9 mm., 35 days conception age. Note milk line and primordium of mammary gland under anterior limb bud. The caudal part of the milk line is not visible in the gross. ×8.

Fig. 5. Embryo C 479, C.-R.L. 11.3 mm., 34 days conception age. Cross section of entire embryo, showing milk lines projecting from body wall medial and dorsal to limb buds. Section 45-2-5; haematoxylin and eosin; ×20.

Fig. 6. Same as figure 5; ×300.

Fig. 7. Monkey no. 34. Mammary gland of newborn. Gross mount; alum cochineal; ×12.

Fig. 8. Monkey no. 715A. Section of mammary gland of newborn, showing hyperplastic epithelium lining ducts. Haematoxylin and eosin; ×250.

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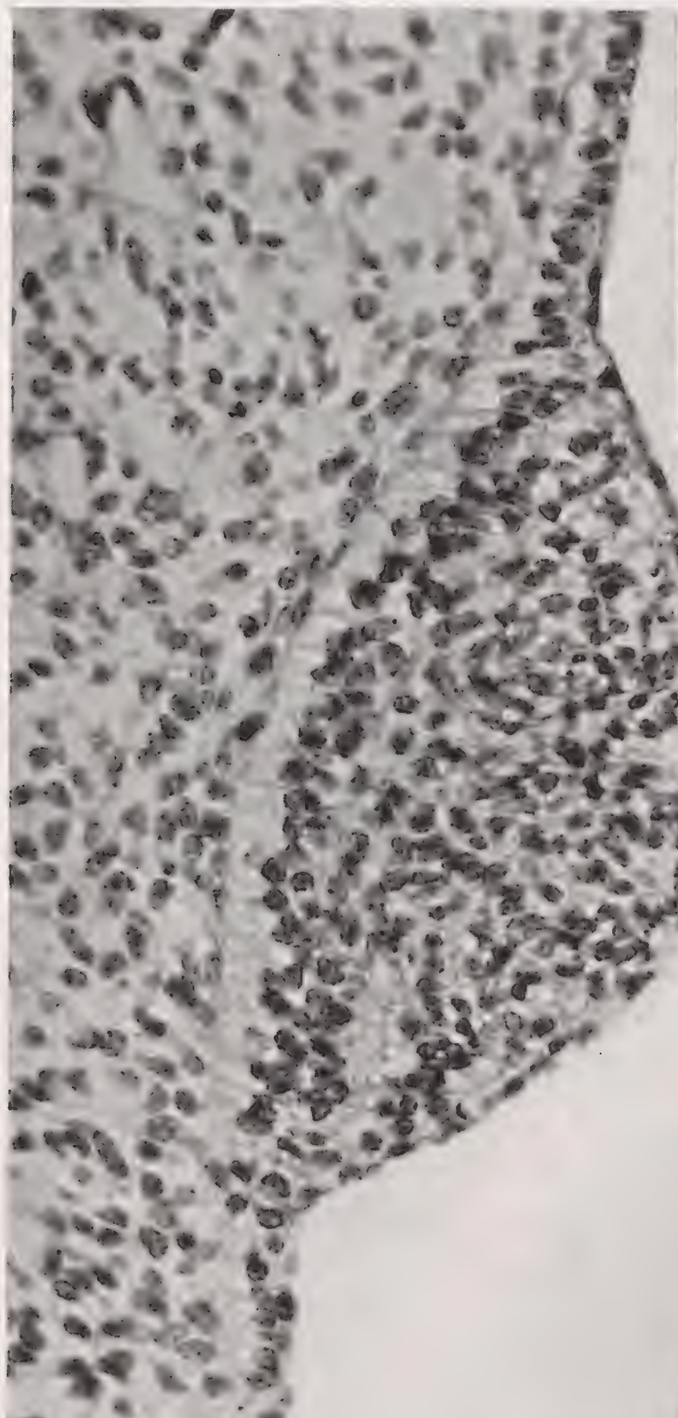

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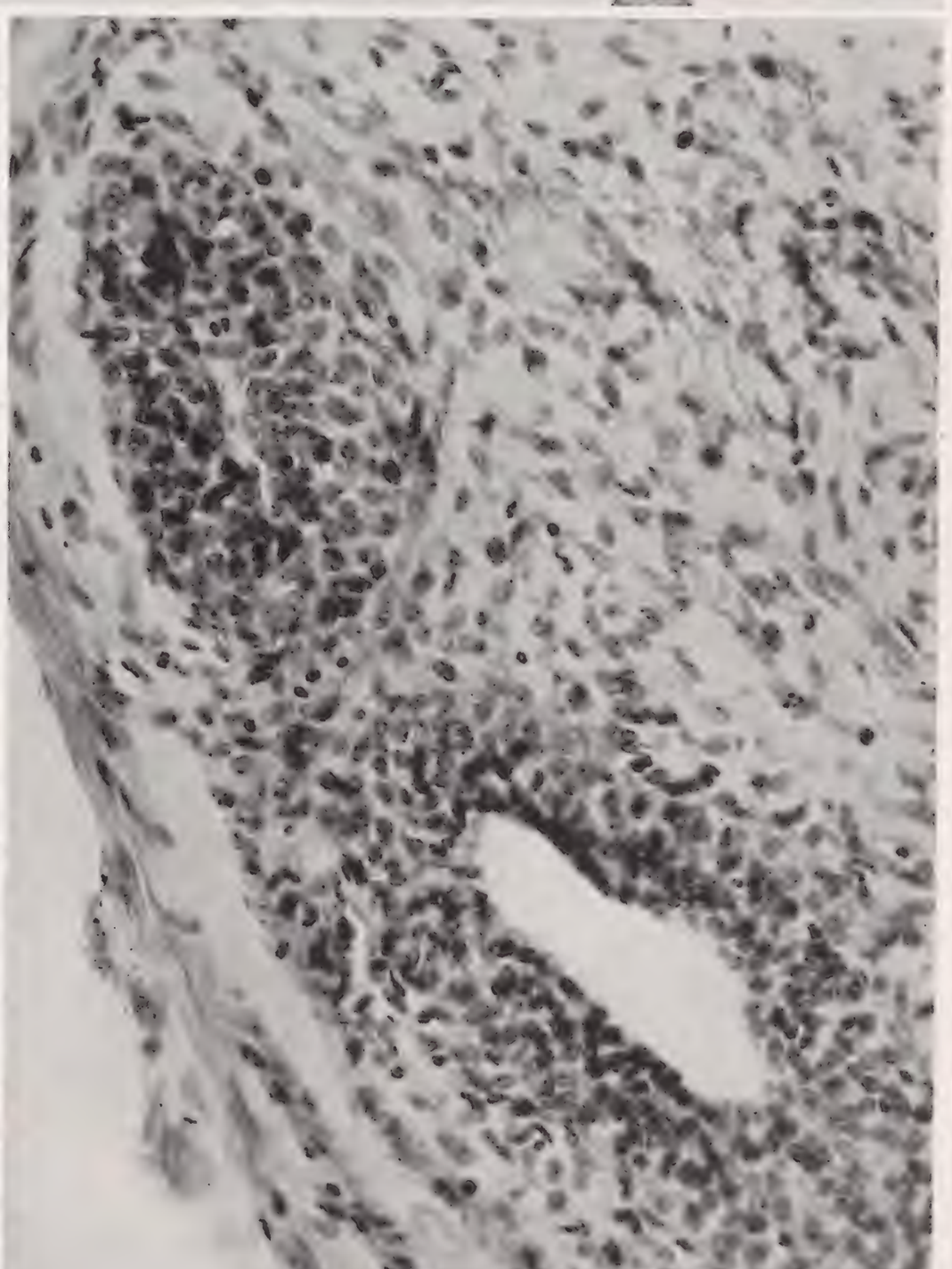

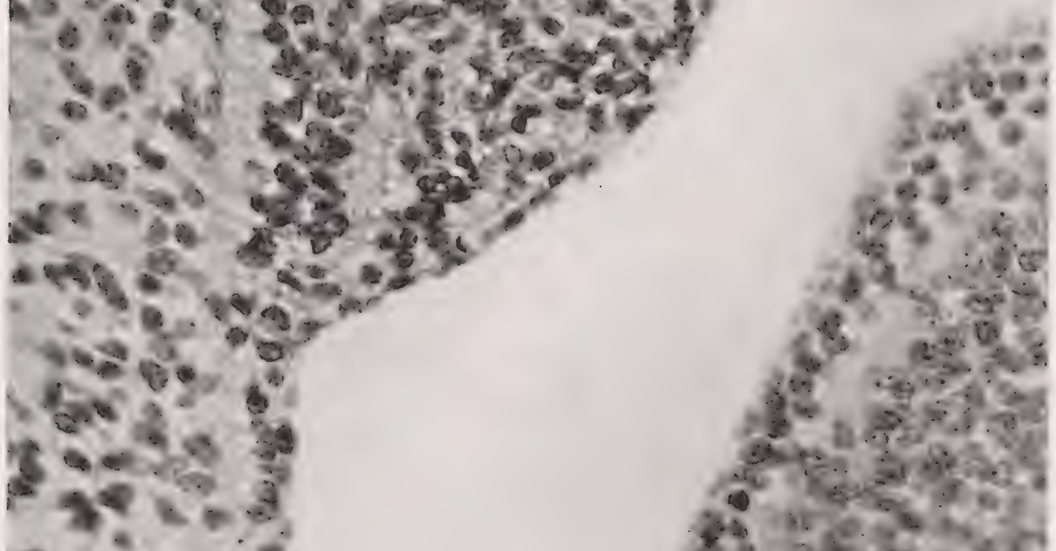

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## Plate 2

Fig. 9. Monkey no. 695. Mammary gland of mature amenorrheic female. Gross mount; alum cochineal; ×1½.

Fig. 10. Monkey no. 604. Mammary gland of mature female following intravascular injection with India ink. Note extreme vascularity of lobules. Delicate capillary network surrounds pale-staining duct crossing center of field. Gross mount; alum cochineal; ×50.

Fig. 11. A lactating monkey.

Figs. 12, 13. Mammary glands of two preadolescent females of similar size, showing range of individual variation in size and development. Gross mounts; alum cochineal; ×2½.

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## Plate 3

Figs. 14, 15. Biopsy specimens of mammary glands of two adult females, obtained 11 days after ovulation. Note individual variation. Gross mounts; alum cochineal; ×12.

Fig. 16. Monkey no. 118. Typical supernumerary nipple, below right normal nipple.

Fig. 17. Monkey no. 313. Supernumerary mammary gland, consisting of only 2 short ducts, showing relation to lower border of left normal mammary gland. Gross mount; alum cochineal; ×3. The incidence of supernumerary mammae in the Carnegie colony was about 1.4 per cent.

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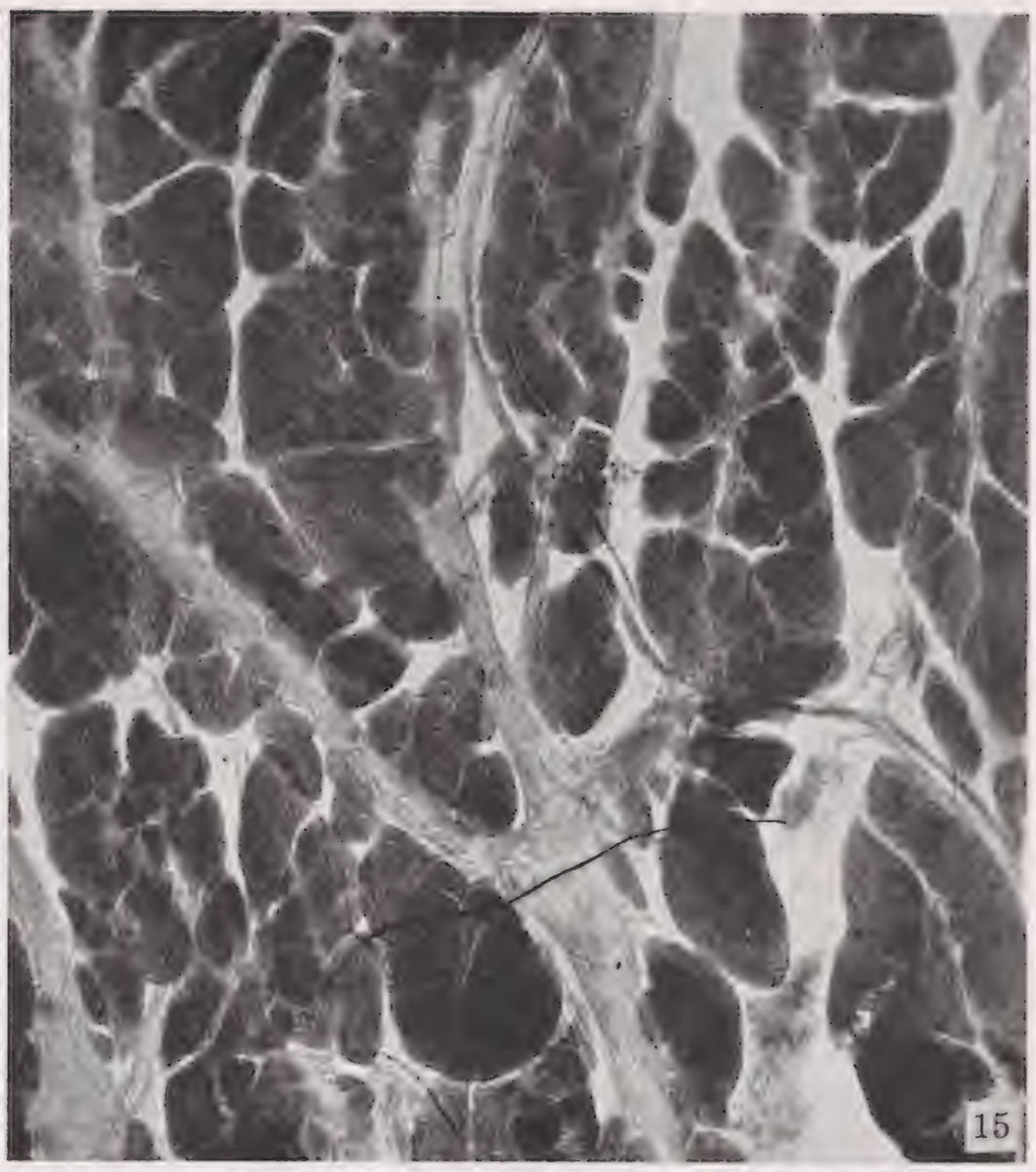

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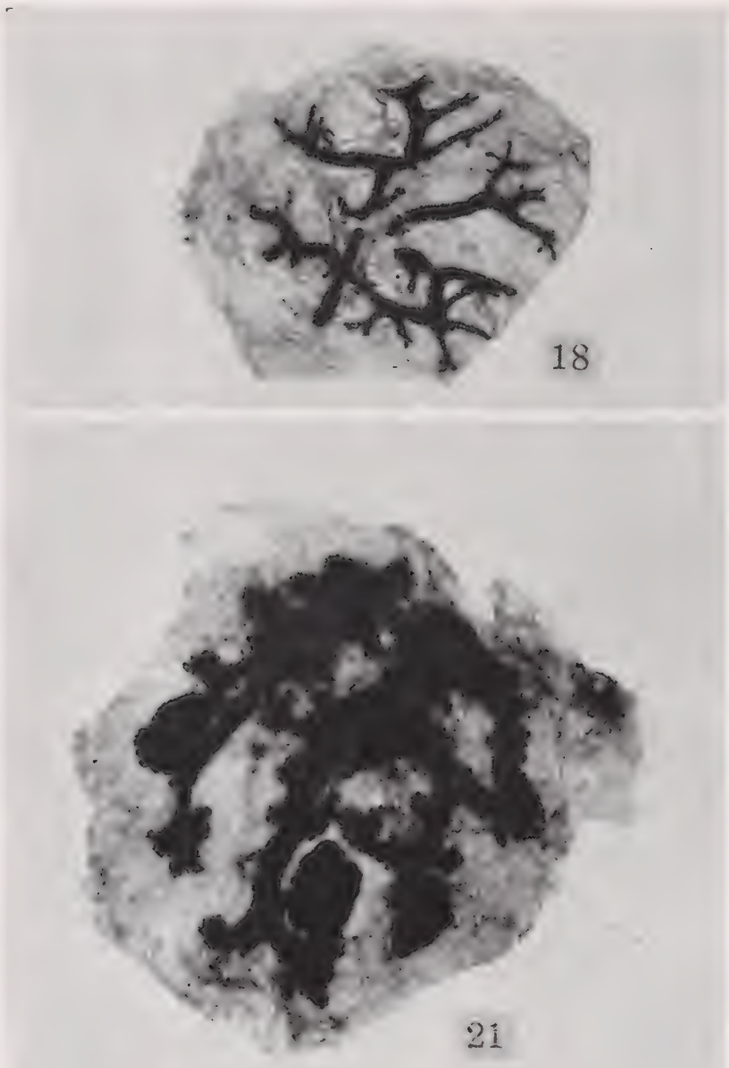
## Plate 4

Figs. 18–27. Mammary glands of 10 mature male monkeys, selected from a group of 24. Note individual variation. Gross mounts; alum cochineal; ×3.



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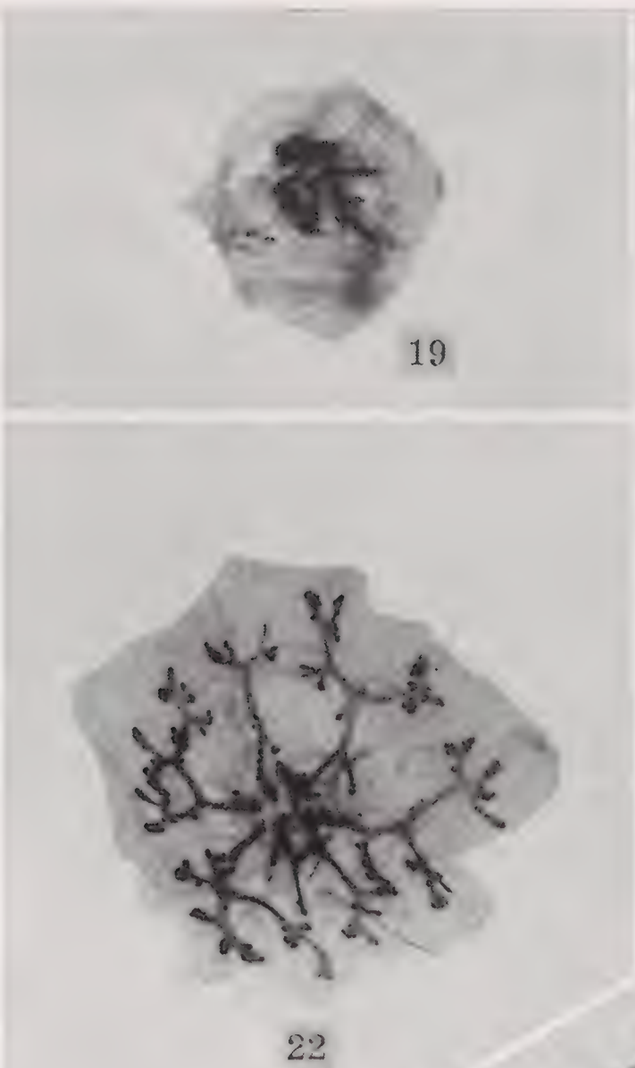

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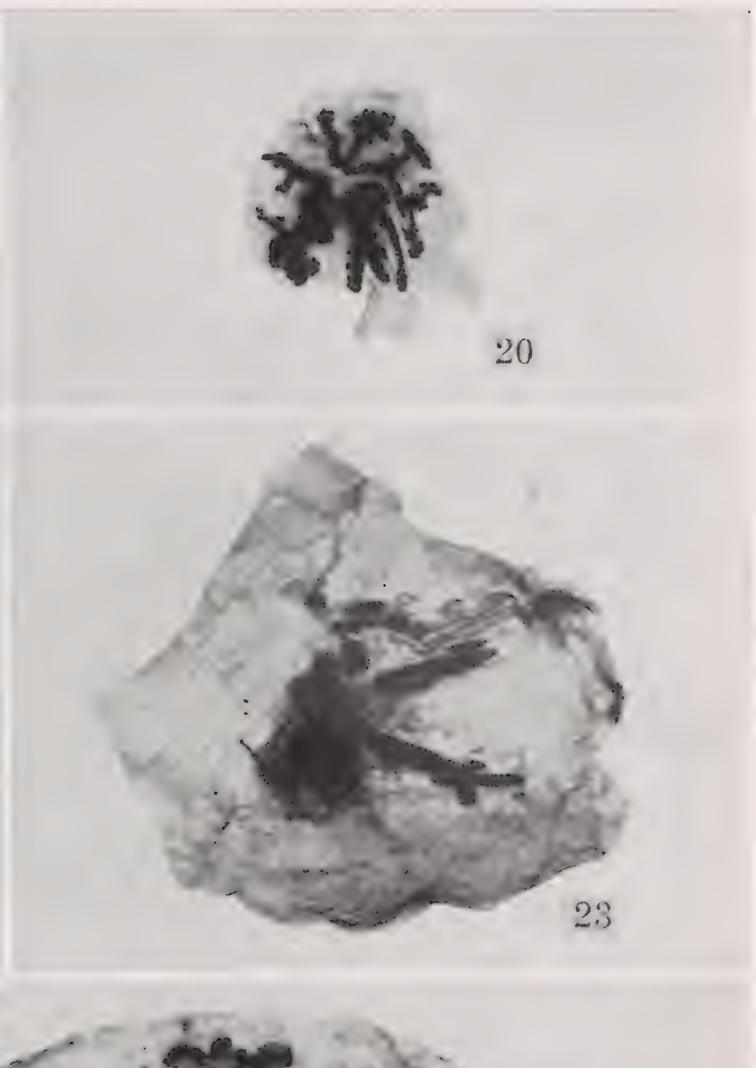

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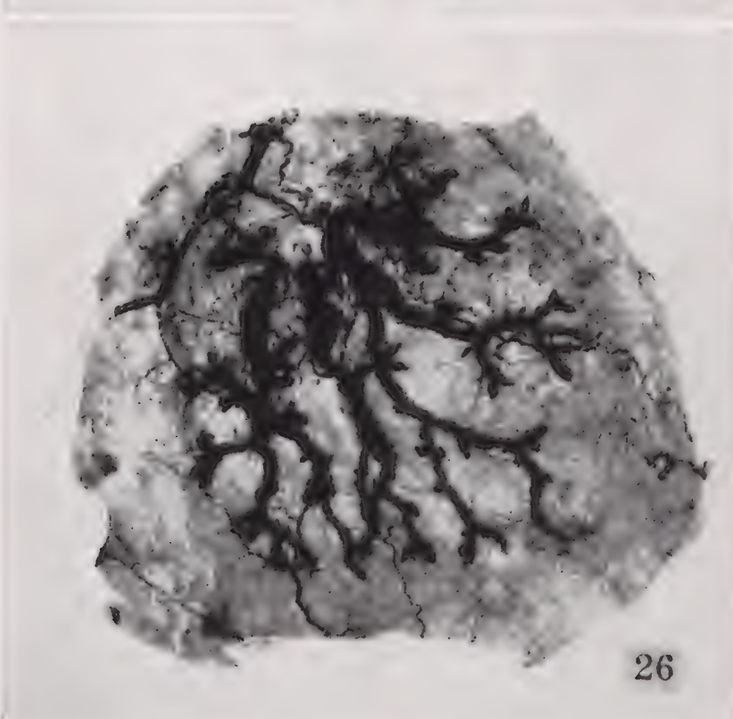

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## Plate 5

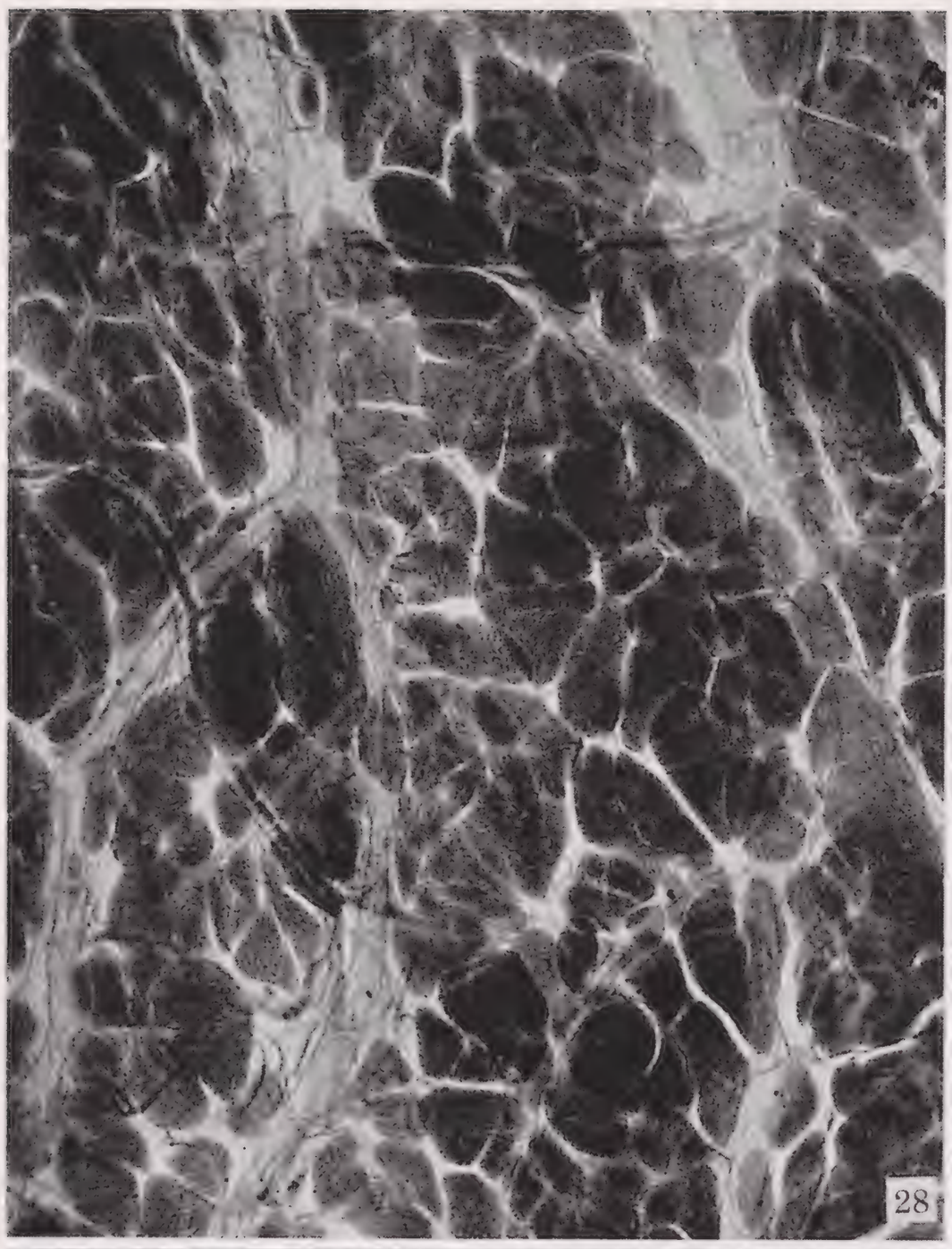
Figs. 28–31. Monkey no. 612. Biopsy specimens of mammary glands during two successive menstrual cycles, contrasting ovulatory and nonovulatory types. Gross mounts; alum cochineal; ×12.

Fig. 28. Day 24 of ovulatory cycle, 11 days after ovulation. Lobules are enlarging. Note contrast with figures 30 and 31.

Fig. 29. Day 2 of succeeding cycle (previous cycle 33 days), showing further enlargement of lobules and dilatation of alveoli.

Fig. 30. Day 17 of nonovulatory cycle, showing regression of lobules and shrinkage of alveoli.

Fig. 31. Day 1 of succeeding cycle (previous cycle 24 days). No change. Contrast with figure 29.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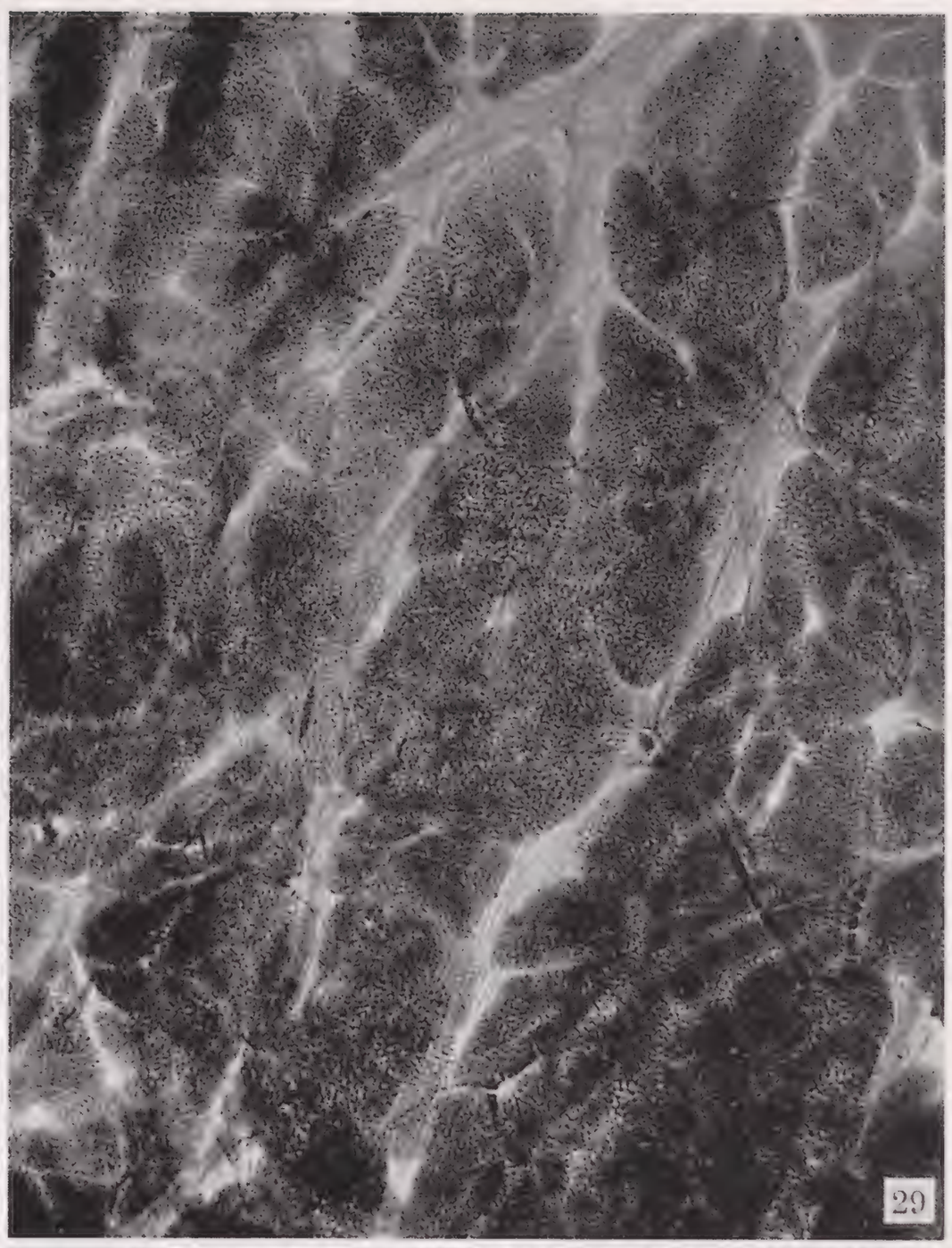
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## Plate 6

Figs. 32–34. Monkey no. 646. Biopsy specimens, showing effect of removal of corpus luteum. Gross mounts; alum cochineal; ×12.

Fig. 32. Day 21 of menstrual cycle, 5 days after ovulation. Lobules are well developed and alveoli distended. Note spongy appearance.

Fig. 33. Five days after surgical removal of ovary containing corpus luteum. Note decrease in size of lobules.

Fig. 34. Seven days later, 12 days after operation. Further shrinkage of the lobules has occurred.

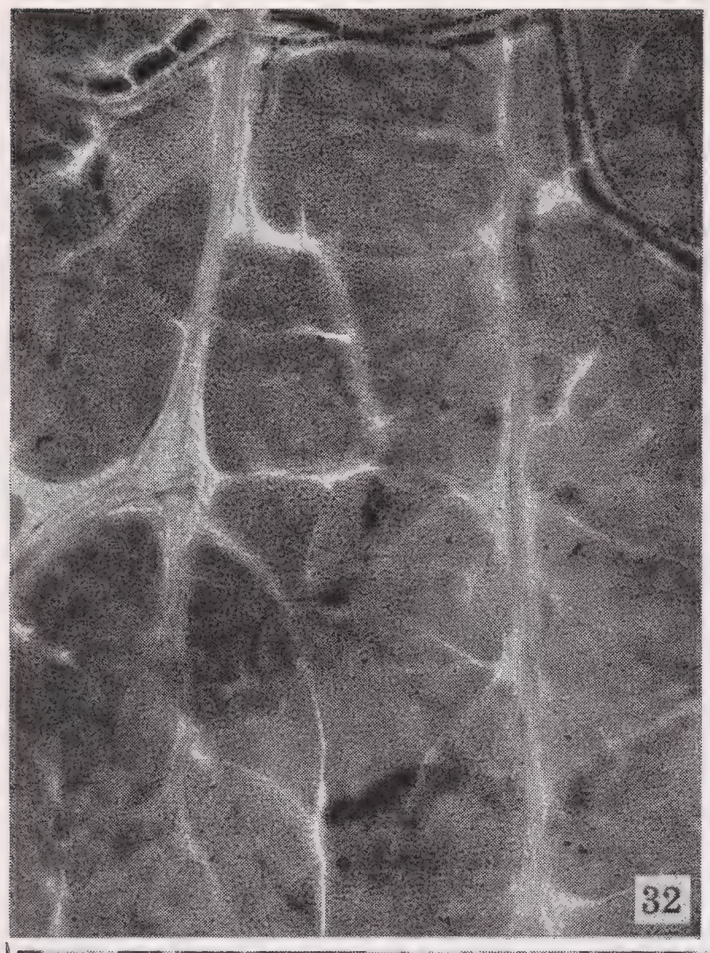
Figs. 35–38. Monkey no. 601. Mammary biopsy specimens at various stages of pregnancy. Gross mounts; alum cochineal; ×12.

Fig. 35. 33 days.

Fig. 36. 68 days.

Fig. 37. 97 days.

Fig. 38. 144 days (parturition).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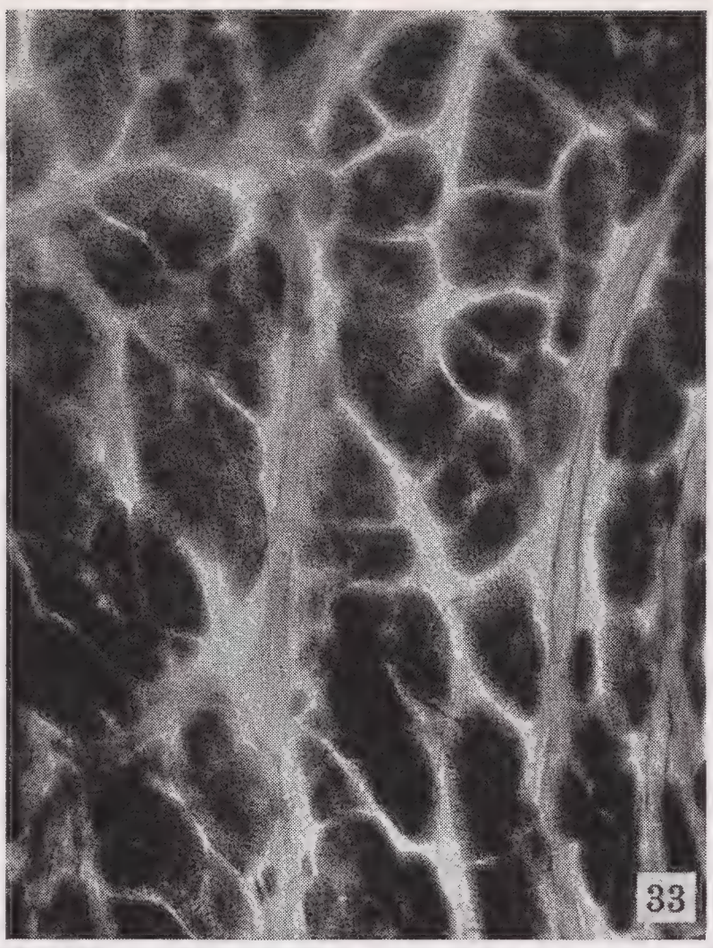
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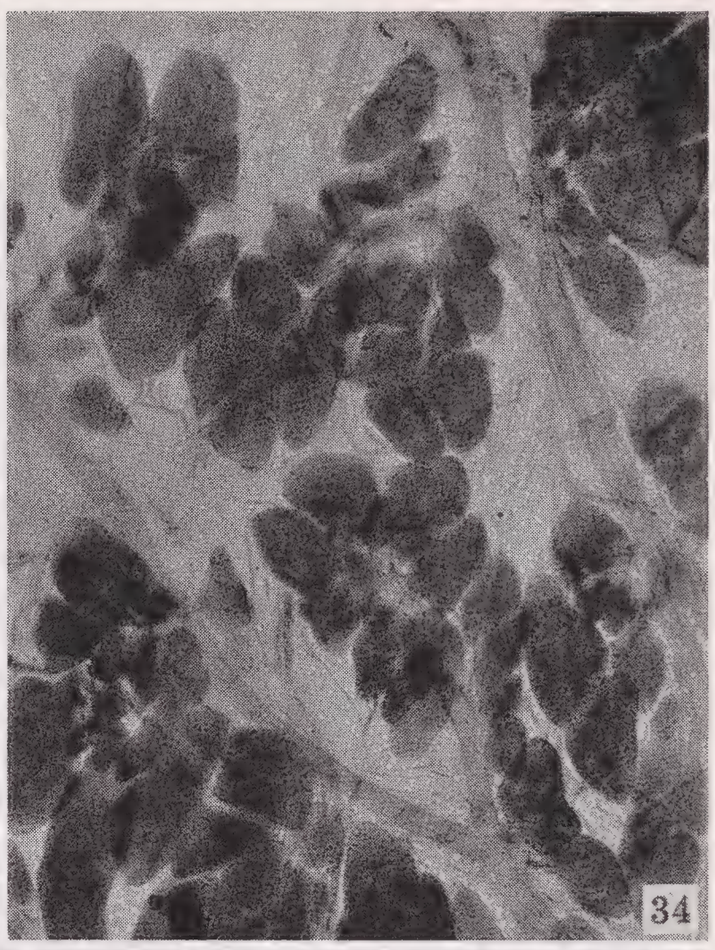
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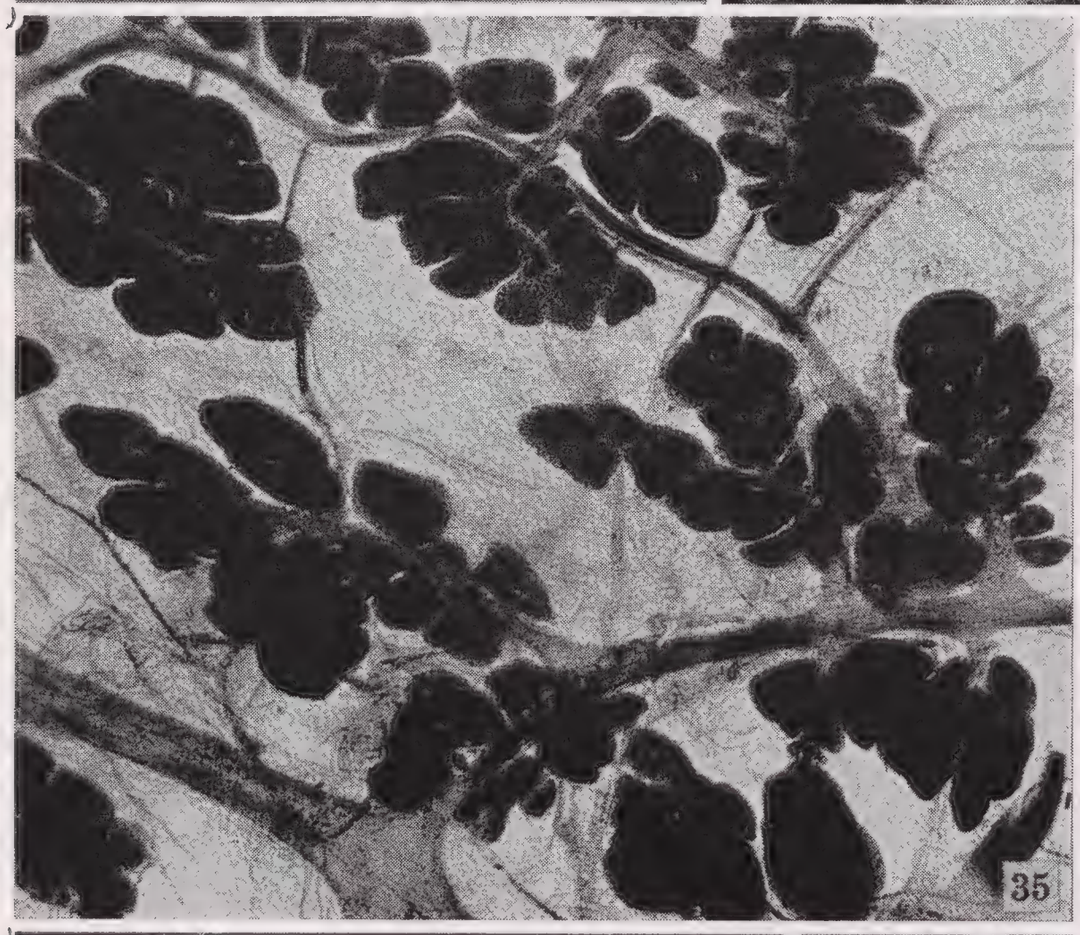
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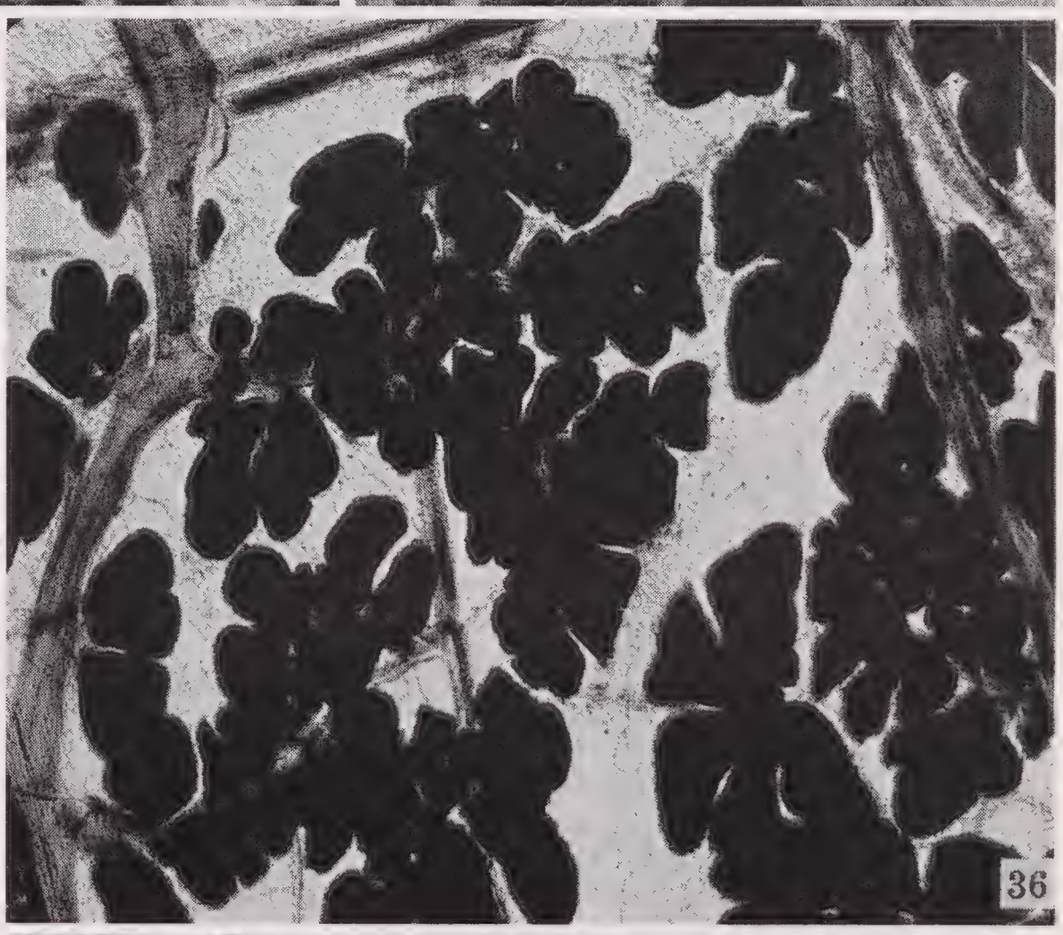
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## Plate 7

Figs. 39–42. Monkey no. 654. Mammary biopsy specimens at various stages of pregnancy. Haematoxylin and eosin; ×100.

Fig. 39. 23 days.

Fig. 40. 95 days.

Fig. 41. 128 days.

Fig. 42. 157 days.

Fig. 43. Same as figure 42, showing mitotic figures. Iron haematoxylin; ×1500.

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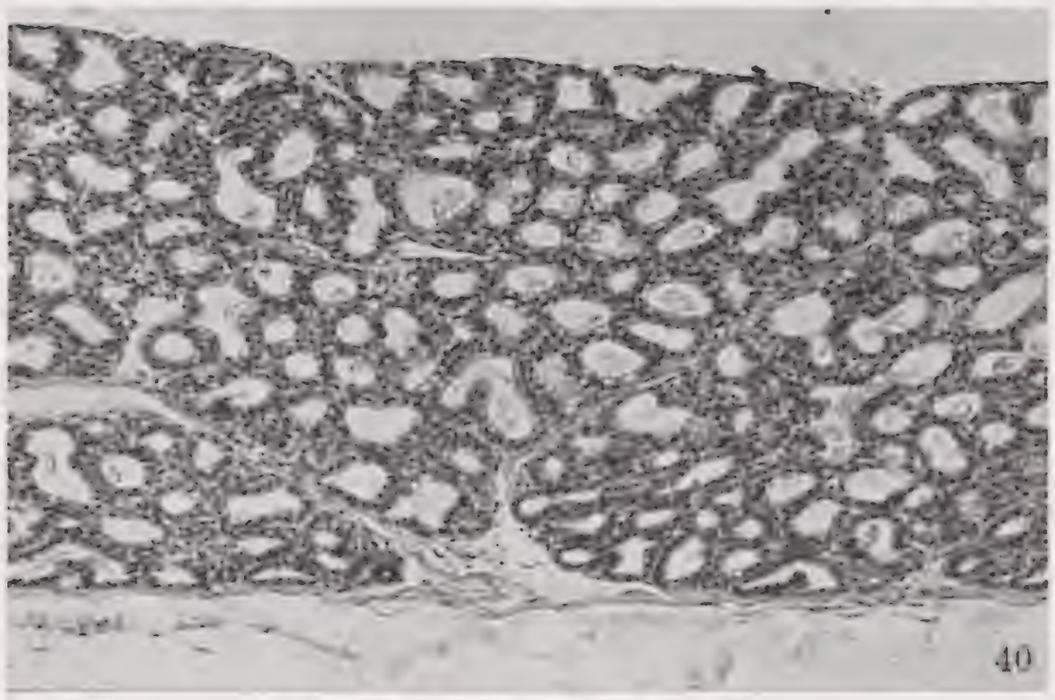
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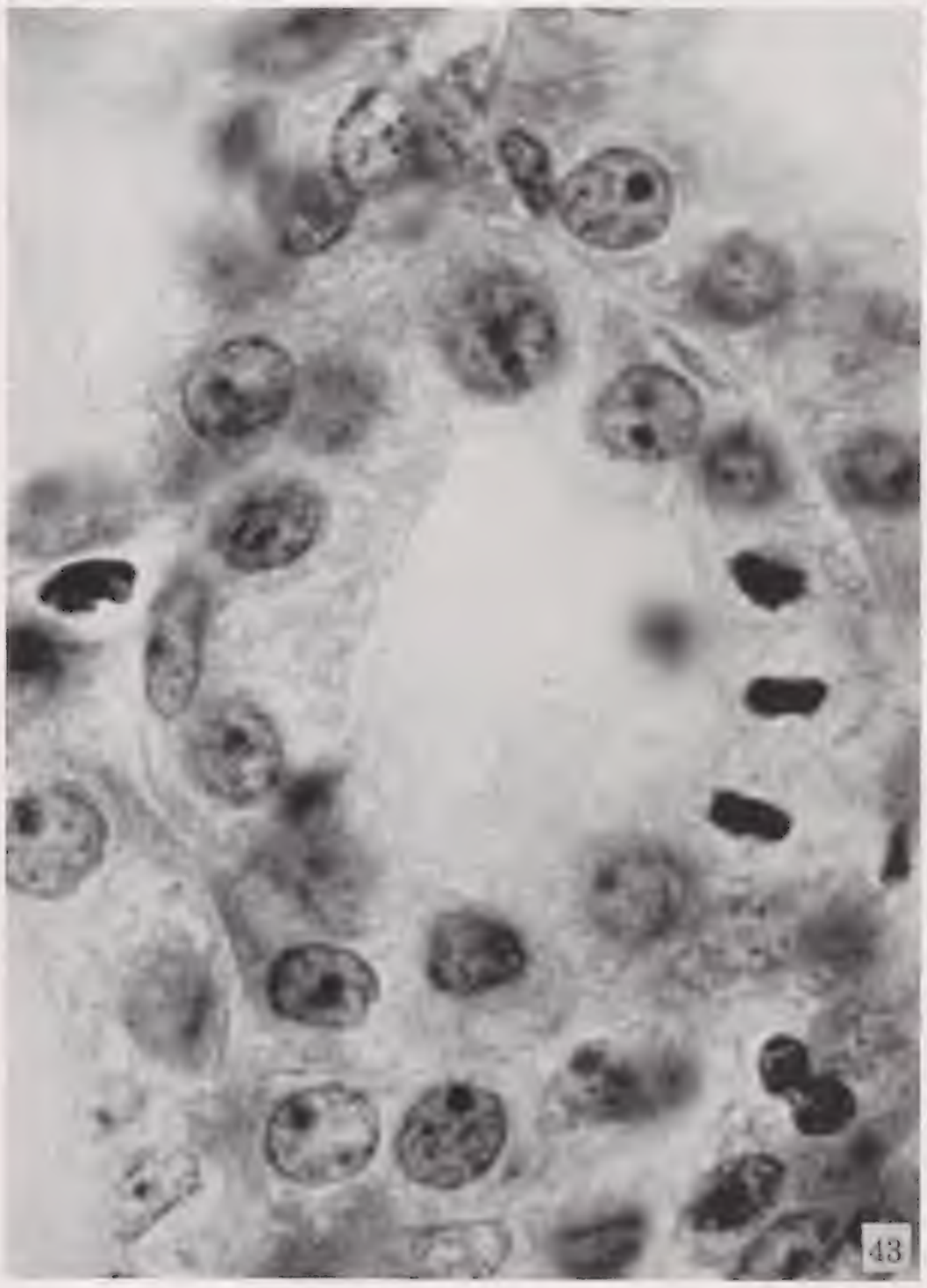
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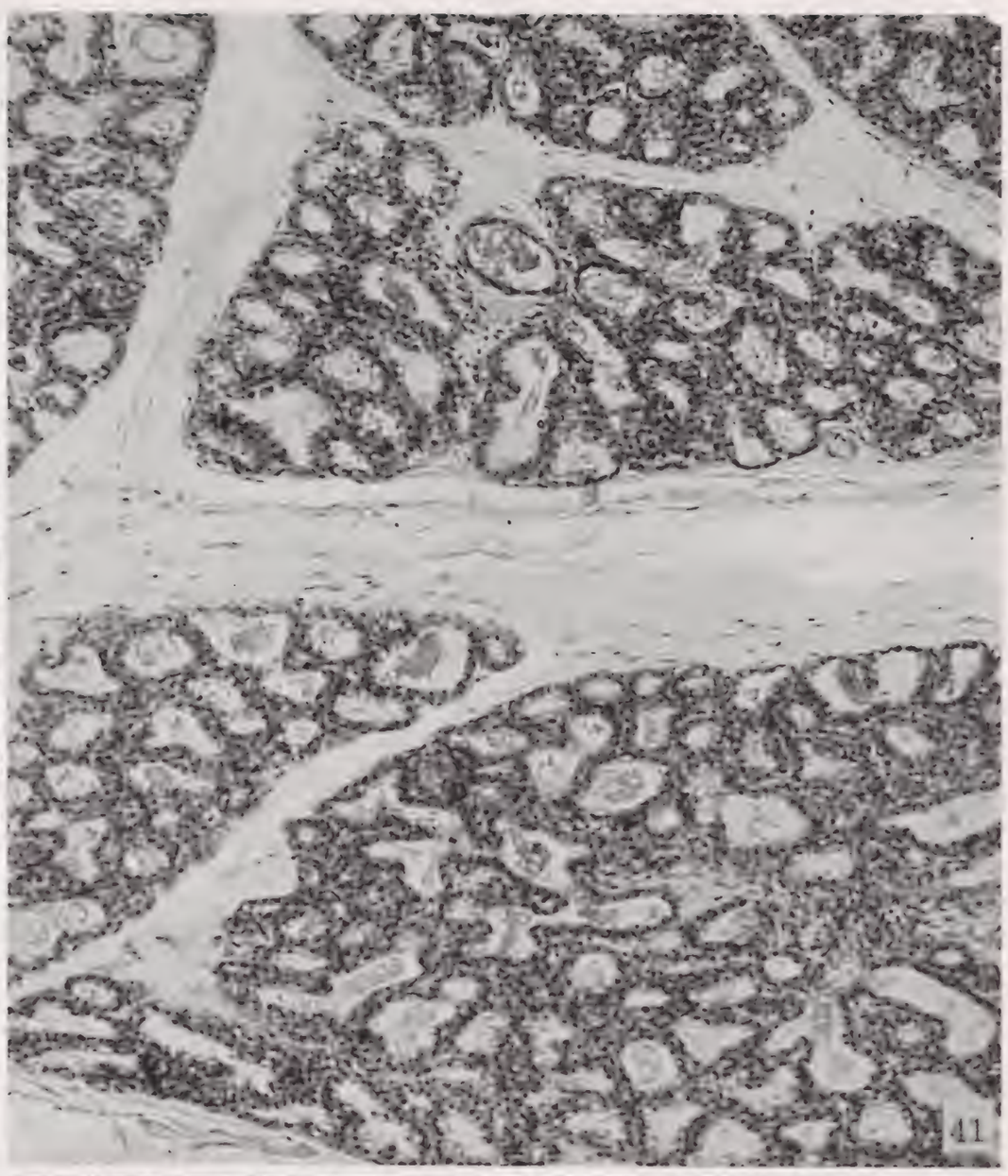
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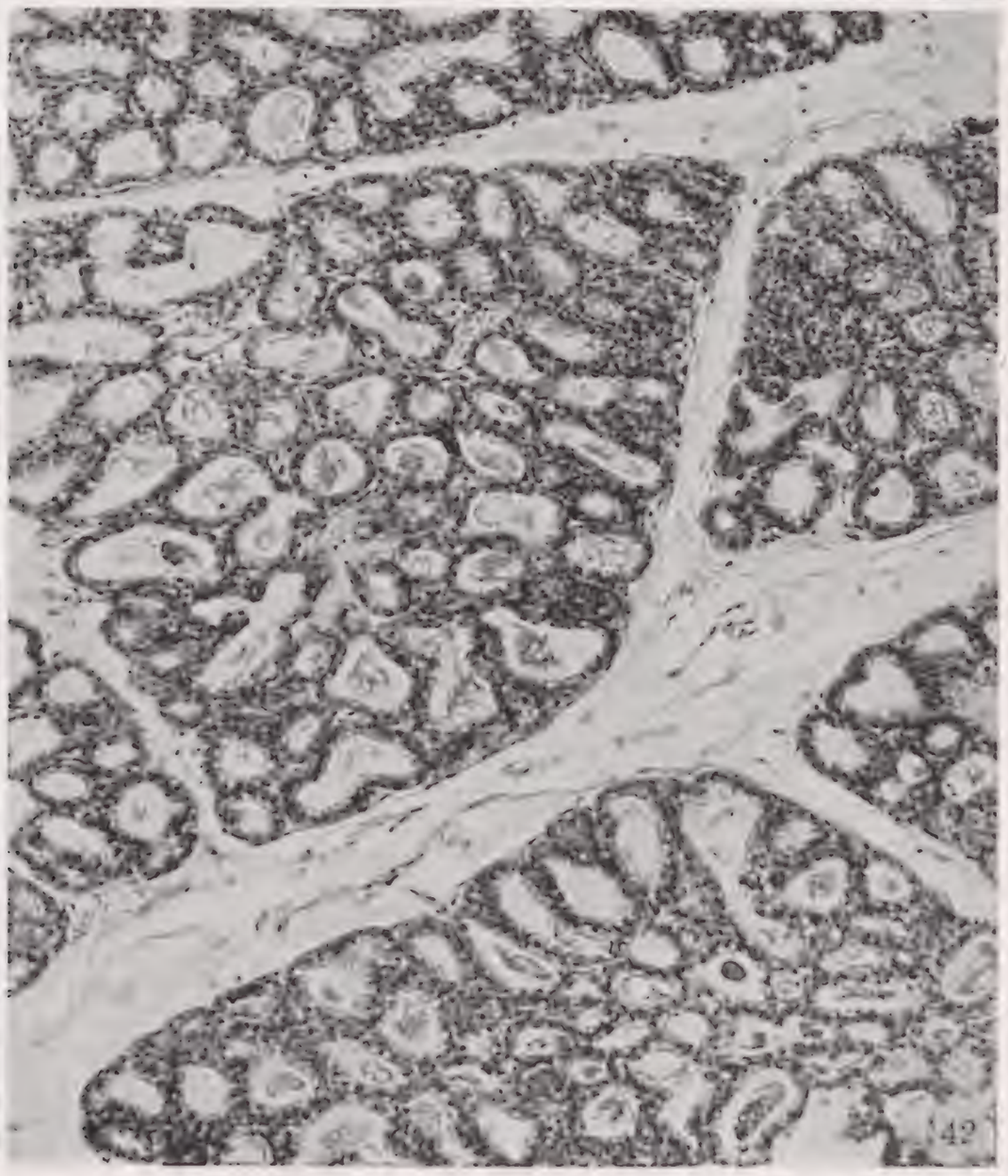
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## Plate 8

Figs. 44, 45. Monkey no. 636. Mammary biopsy specimens before and after artificial interruption of pregnancy at 36 days. Gross mounts; alum cochineal; ×12.

Fig. 44. At 36th day of pregnancy.

Fig. 45. Fourteen days later, after interruption of pregnancy.

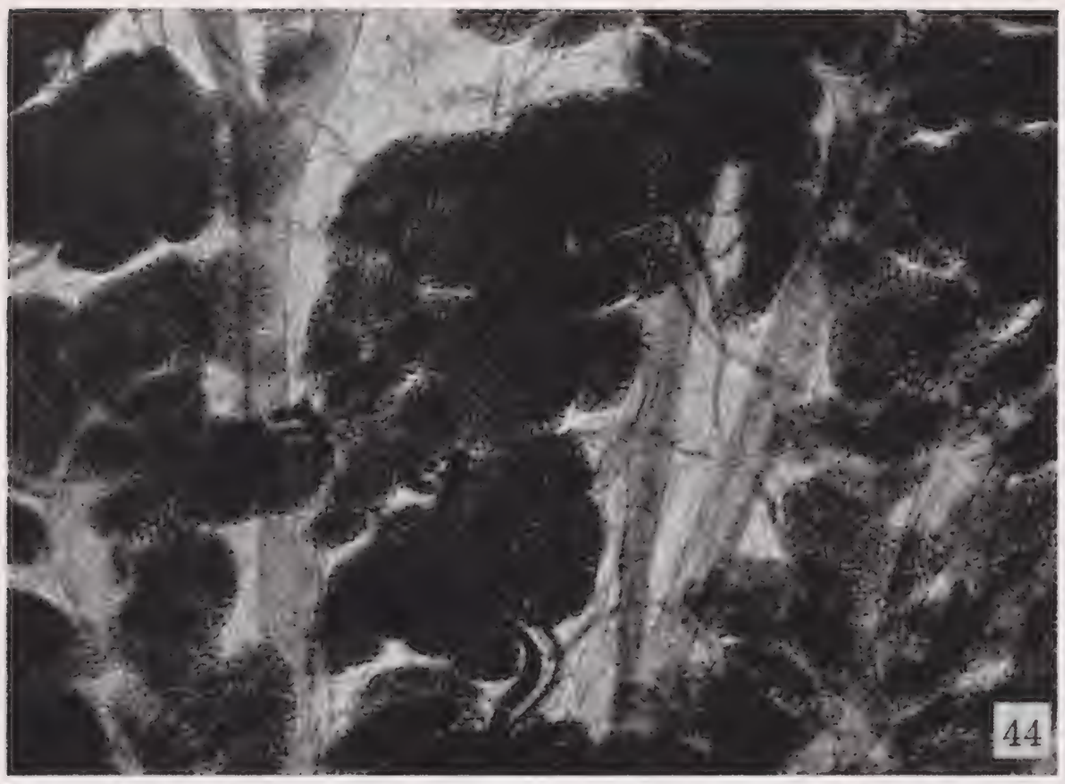
Figs. 46, 47. Monkey no. 680. Mammary biopsy specimens before and after operative abortion at 60 days. Haematoxylin and eosin; ×100.

Fig. 46. At 60th day of pregnancy.

Fig. 47. Two days later, after operative abortion, showing secretory activity of gland.

Fig. 48. Monkey no. 117. Mammary gland of pregnant animal at term, showing effects of recent lactation. Haematoxylin and eosin; ×100.

Fig. 49. Monkey no. 665. Lactating mammary gland, showing lobulated nuclei and binuclear cells. Section 6 microns thick; iron haematoxylin; ×1750.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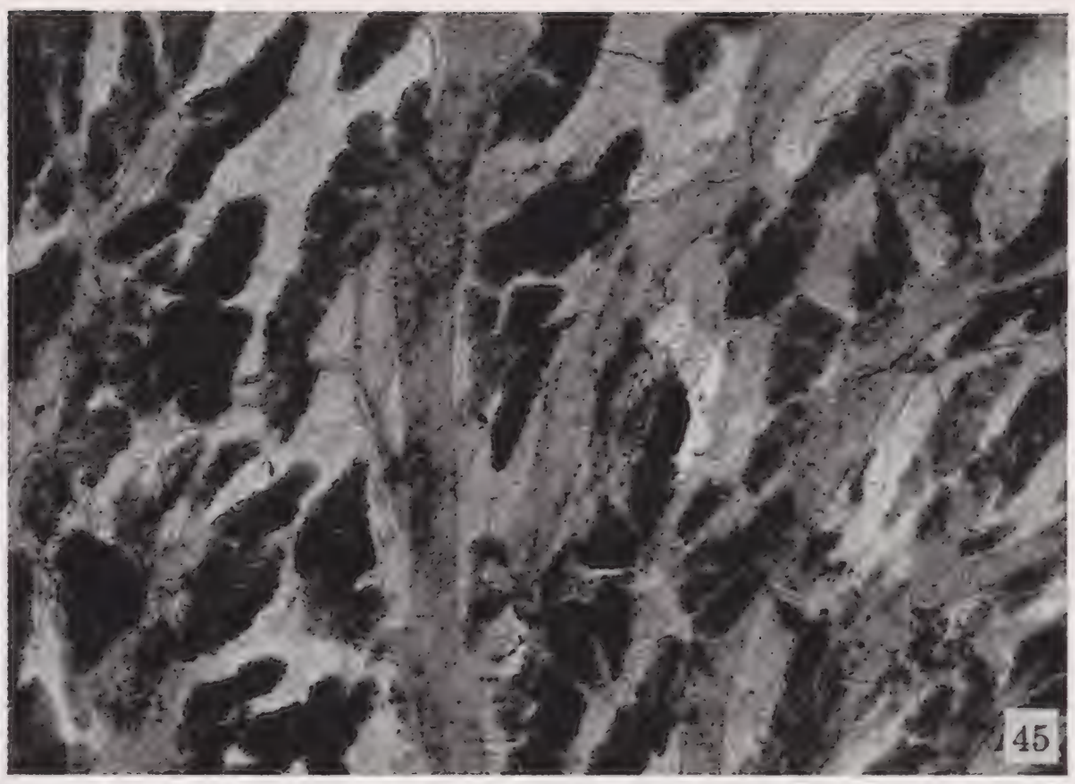
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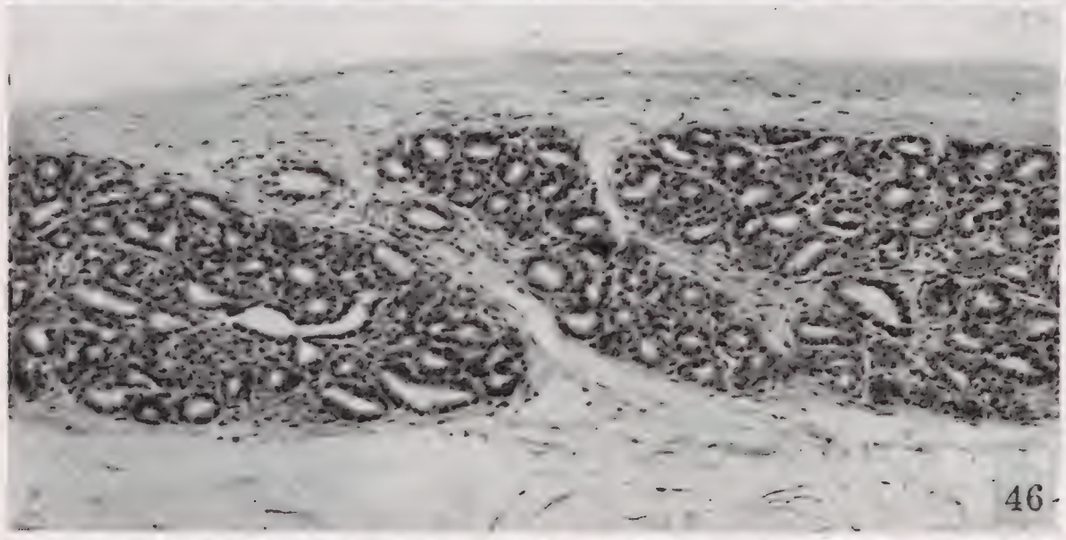
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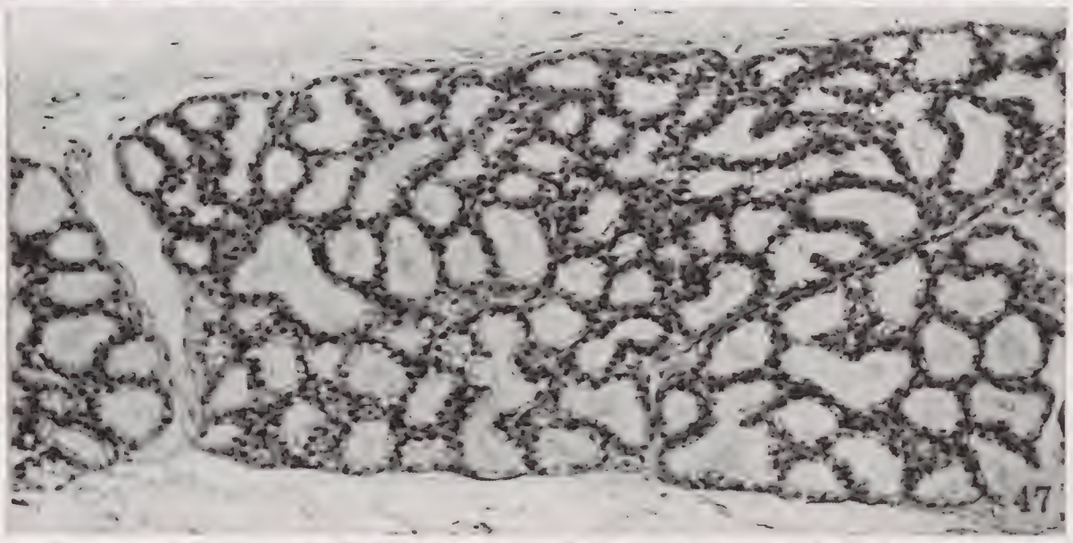
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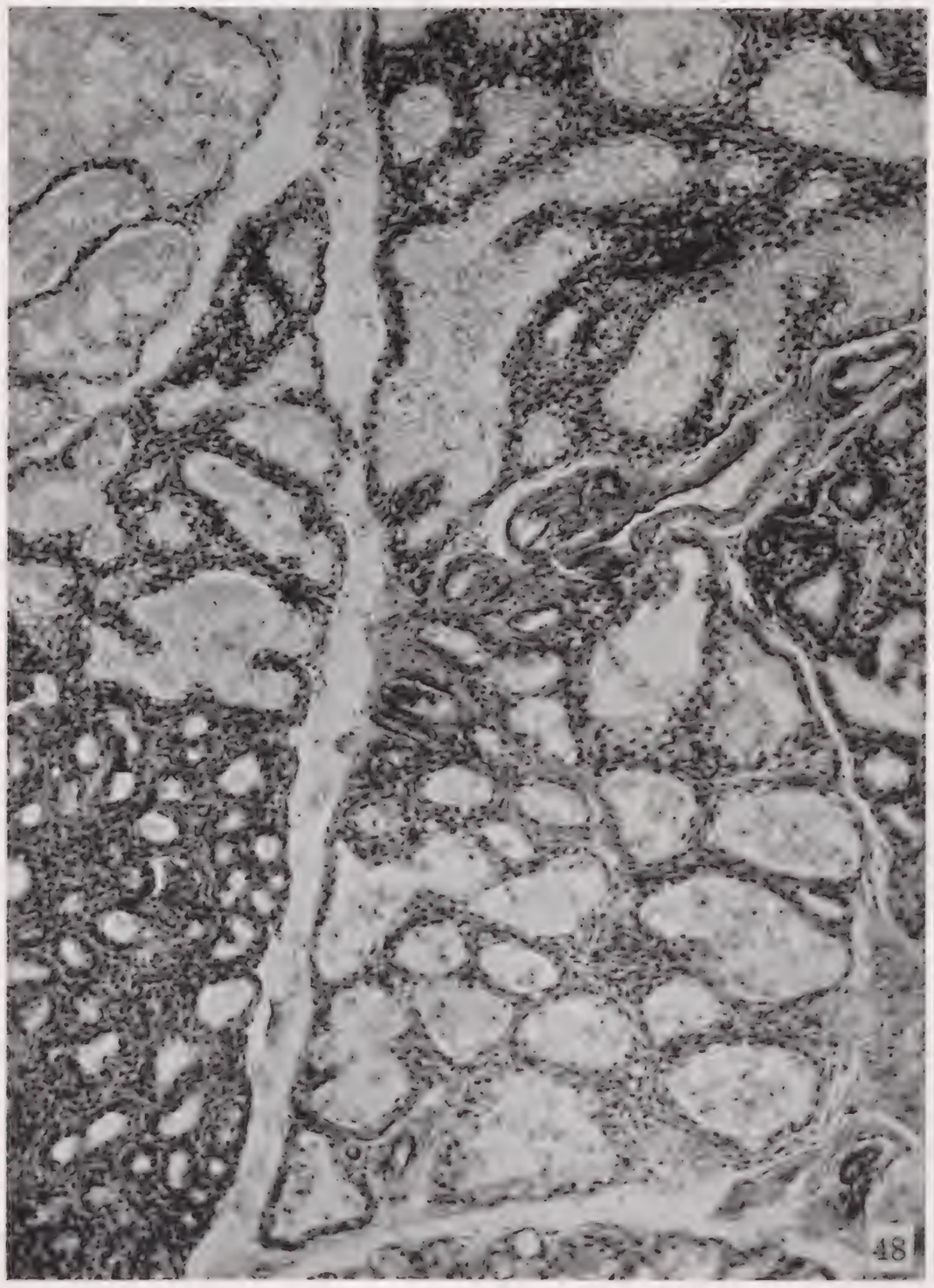
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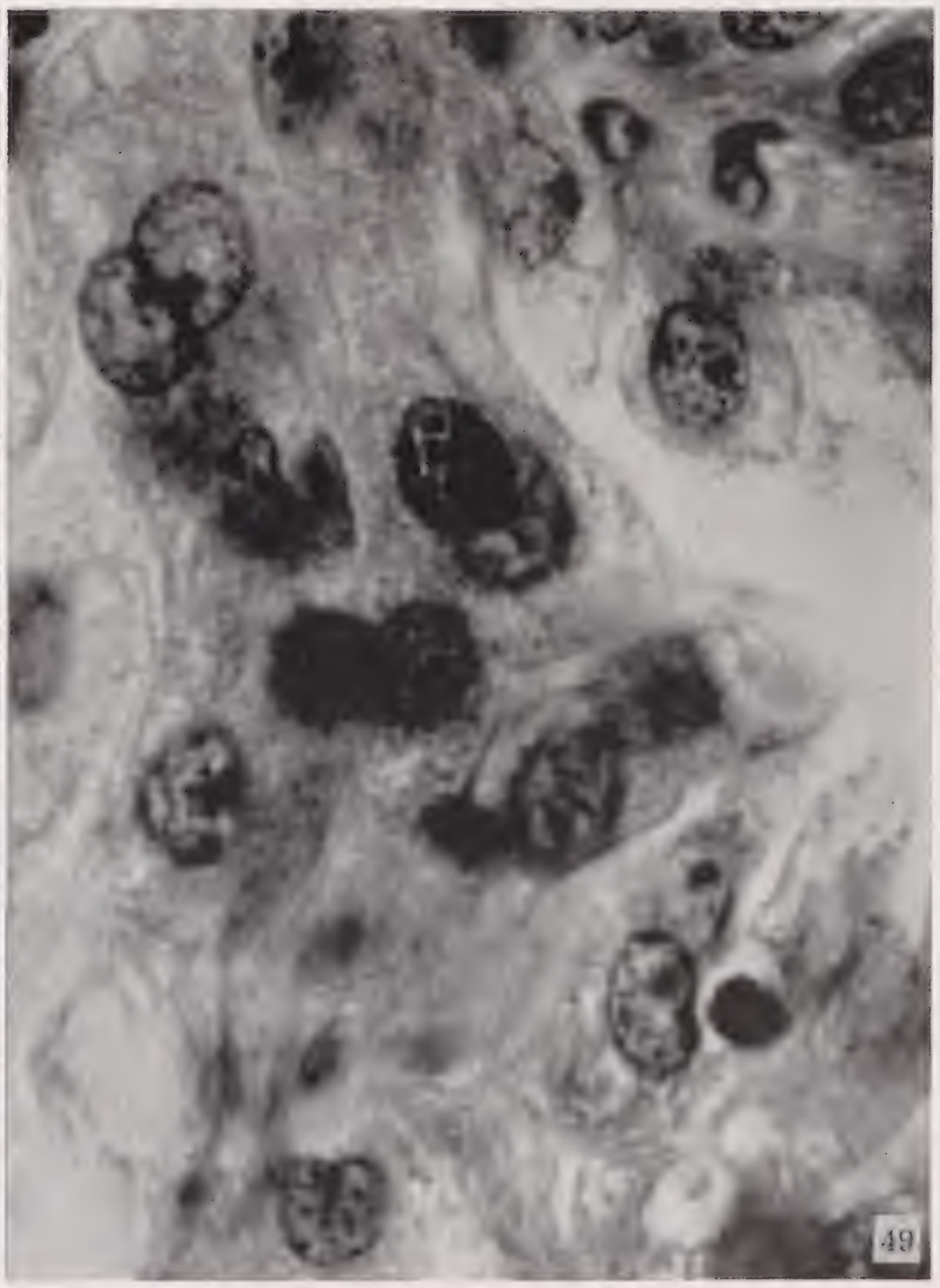
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## Plate 9

Figs. 50, 51. Monkey no. 614. Mammary biopsy specimens, showing involutionary changes in absence of nursing. Gross mounts; alum cochineal; ×12.

Fig. 50. 9 days postpartum.

Fig. 51. 109 days postpartum.

Figs. 52–55. Monkey no. 654. Mammary biopsy specimens at various stages of involution following weaning on 4th postpartum day. Haematoxylin and eosin; ×100.

Fig. 52. At weaning, 4 days postpartum.

Fig. 53. 24 days postpartum.

Fig. 54. 54 days postpartum.

Fig. 55. 87 days postpartum.

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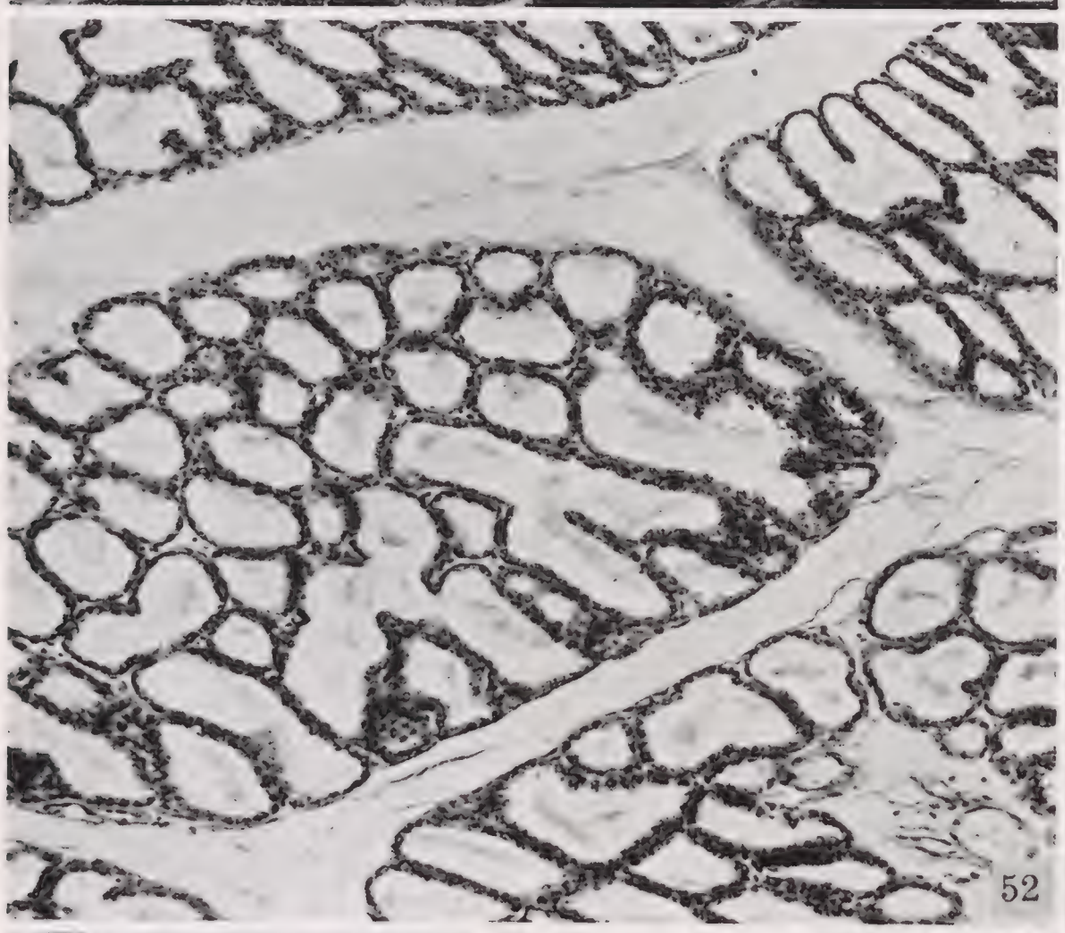
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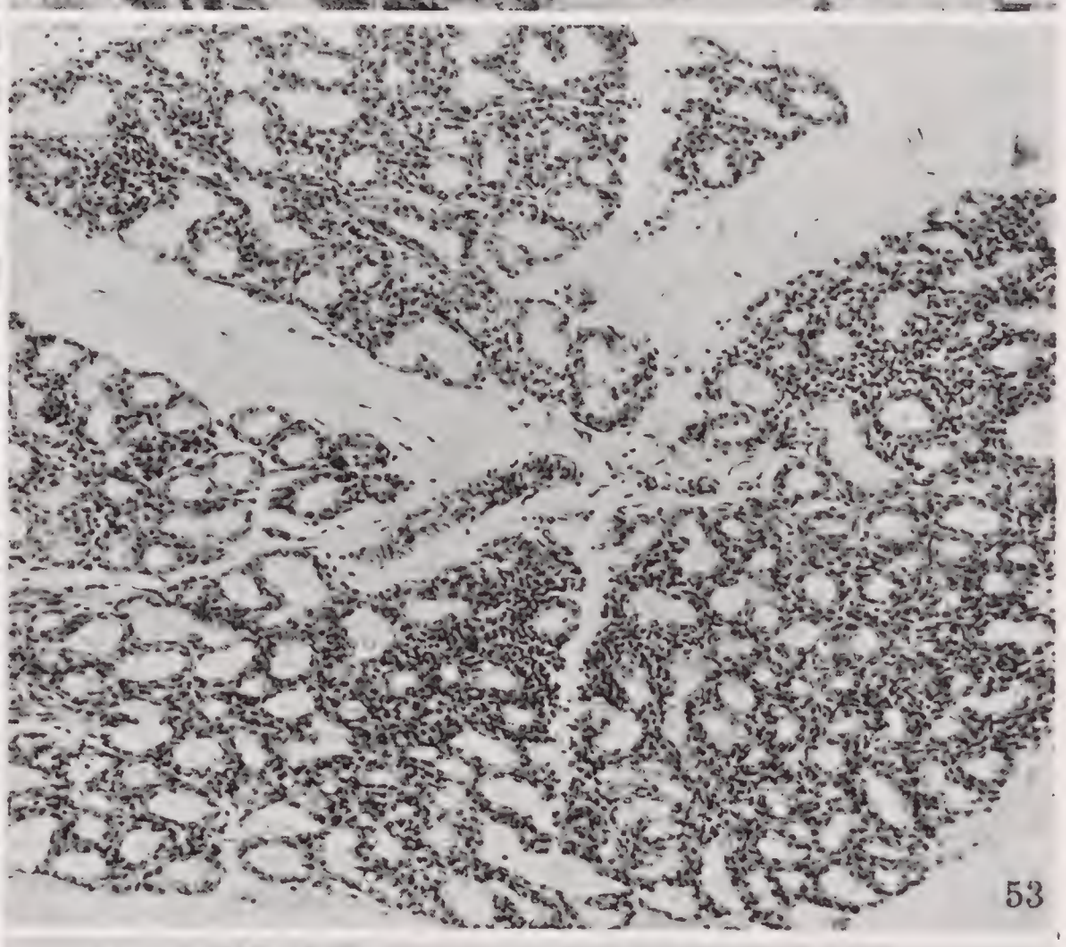
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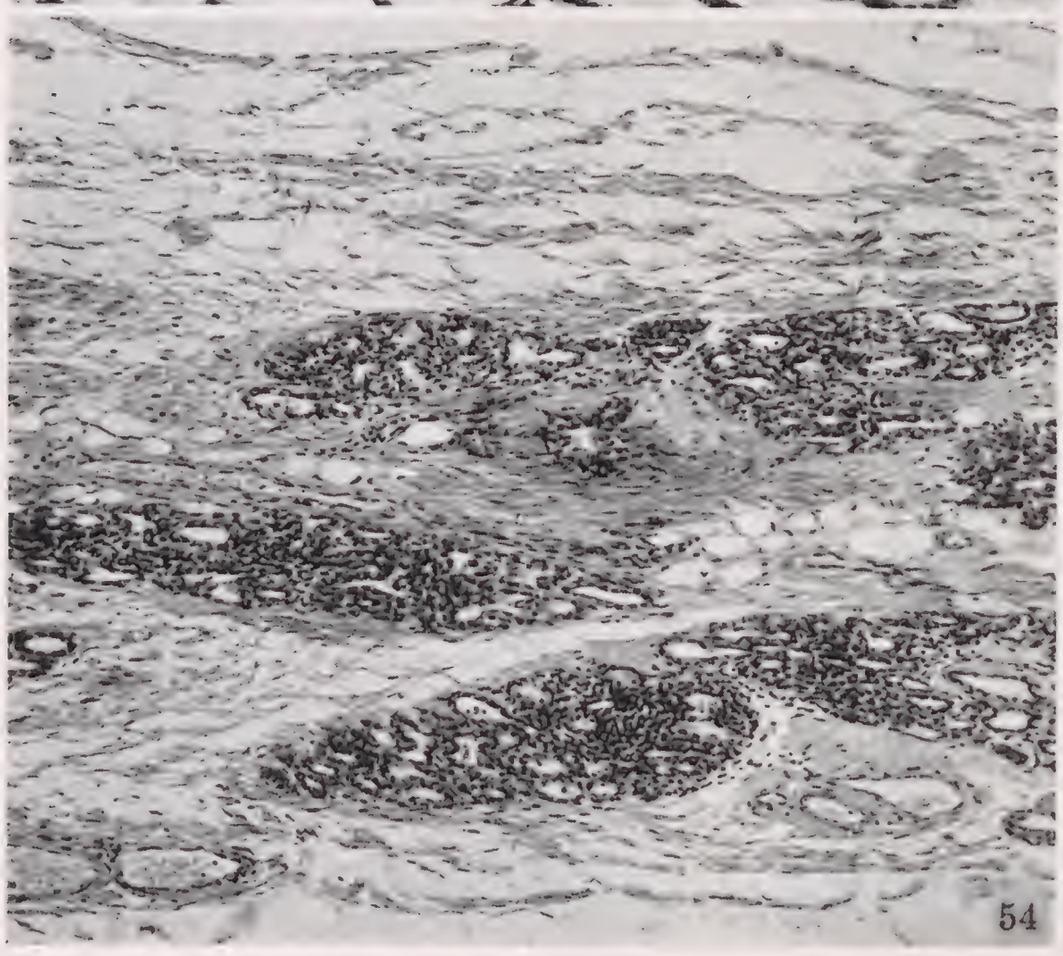
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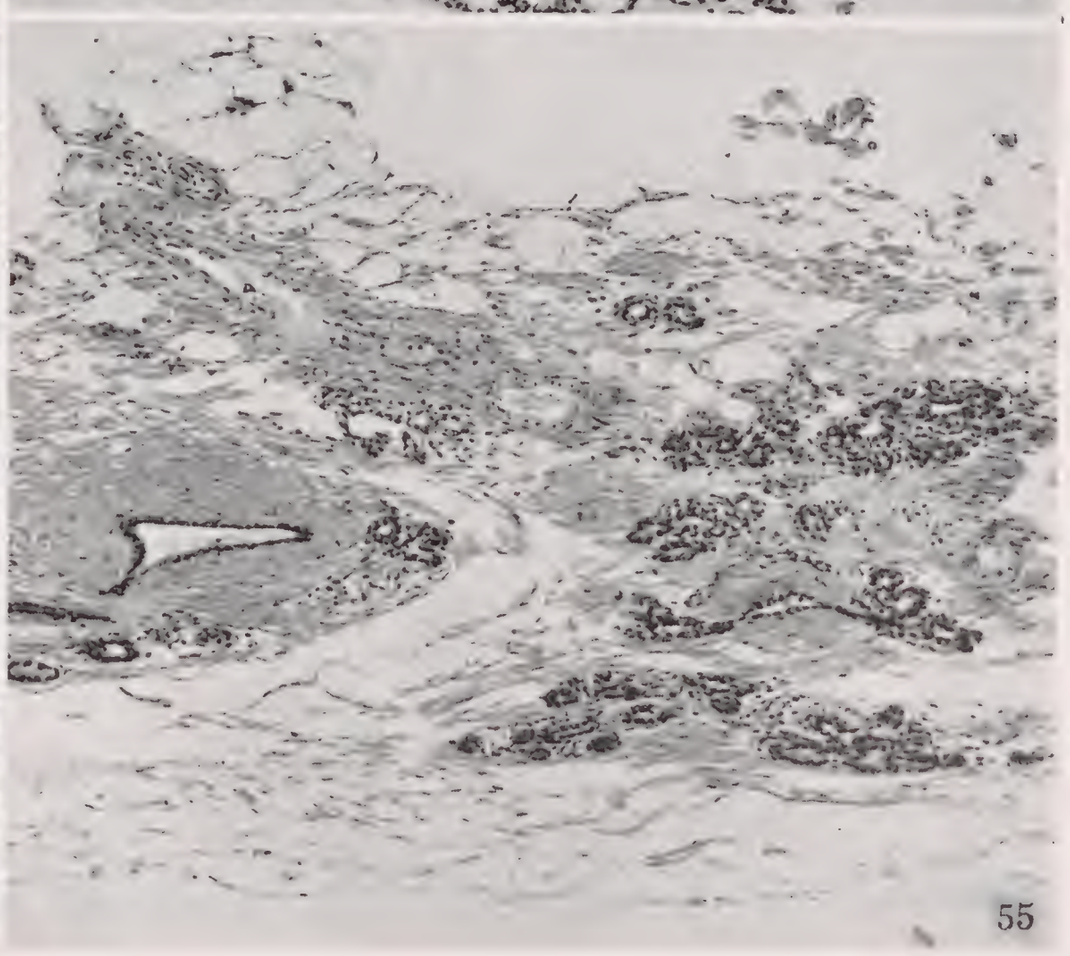
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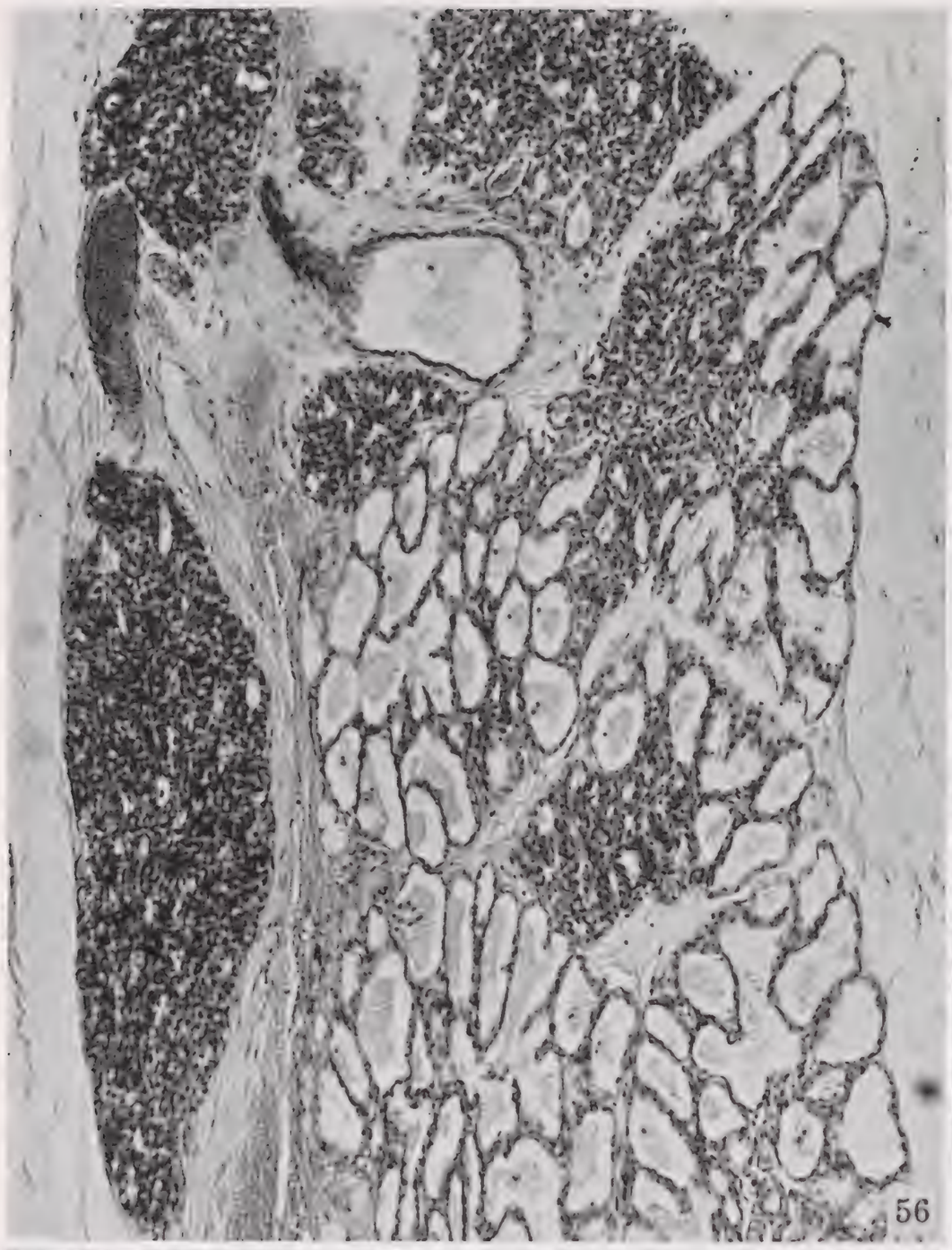
## Plate 10

Fig. 56. Monkey no. 690. Mammary gland on 49th day postpartum, showing variation in rate of involution of different lobules. Haematoxylin and eosin; ×100.

Fig. 57. Monkey no. 652. Mammary gland 13 days after weaning, showing sloughing of alveolar cells into lumen. Haematoxylin and eosin; ×350.

Fig. 58. Monkey no. 614. Mammary gland on 38th day of postpartum involution, showing sloughing of cells lining duct. Haematoxylin and eosin; ×350.

Fig. 59. Monkey no. 652. Section of mammary gland 44 days after weaning, showing leukocytic infiltration of lobule. Iron haematoxylin; ×350.



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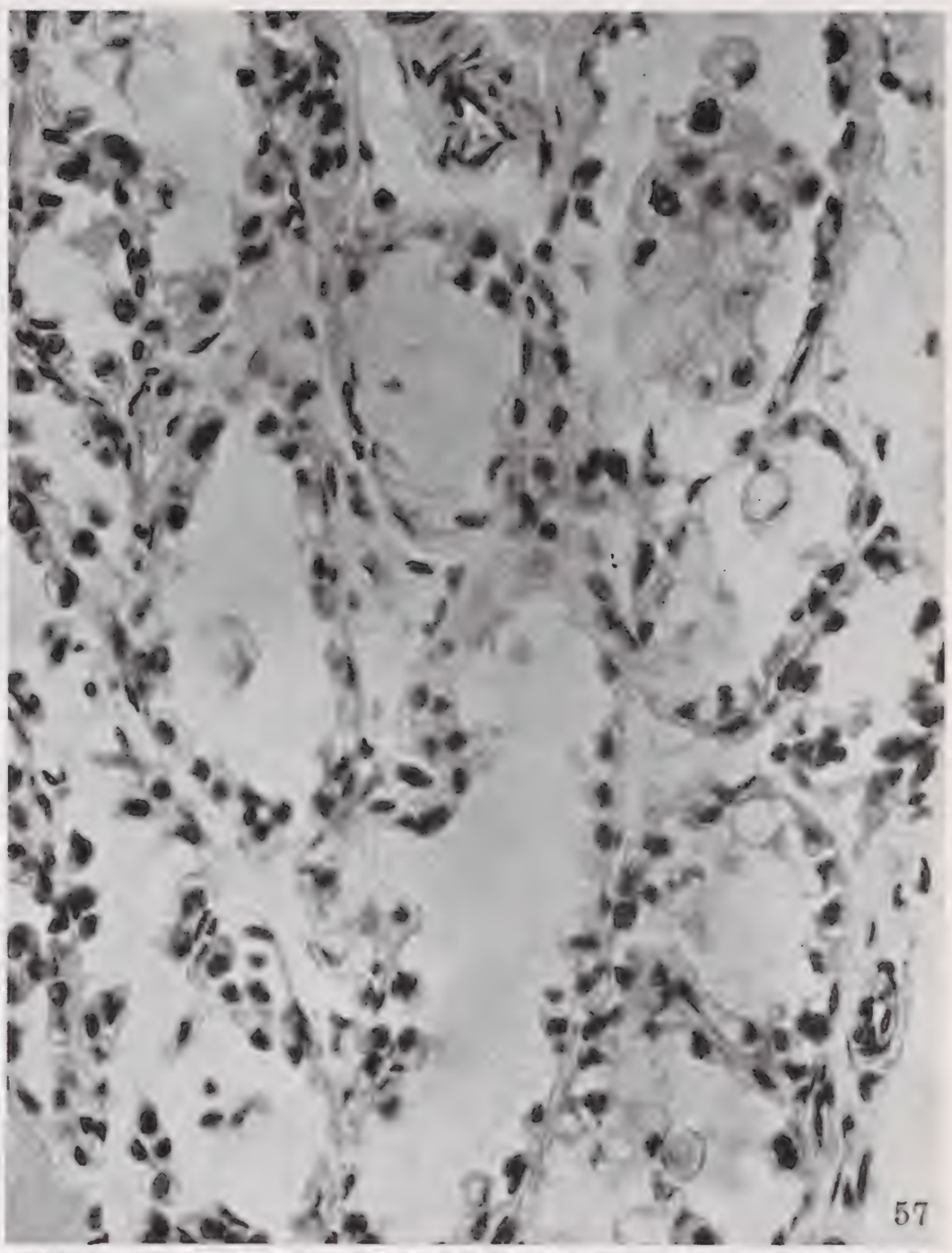

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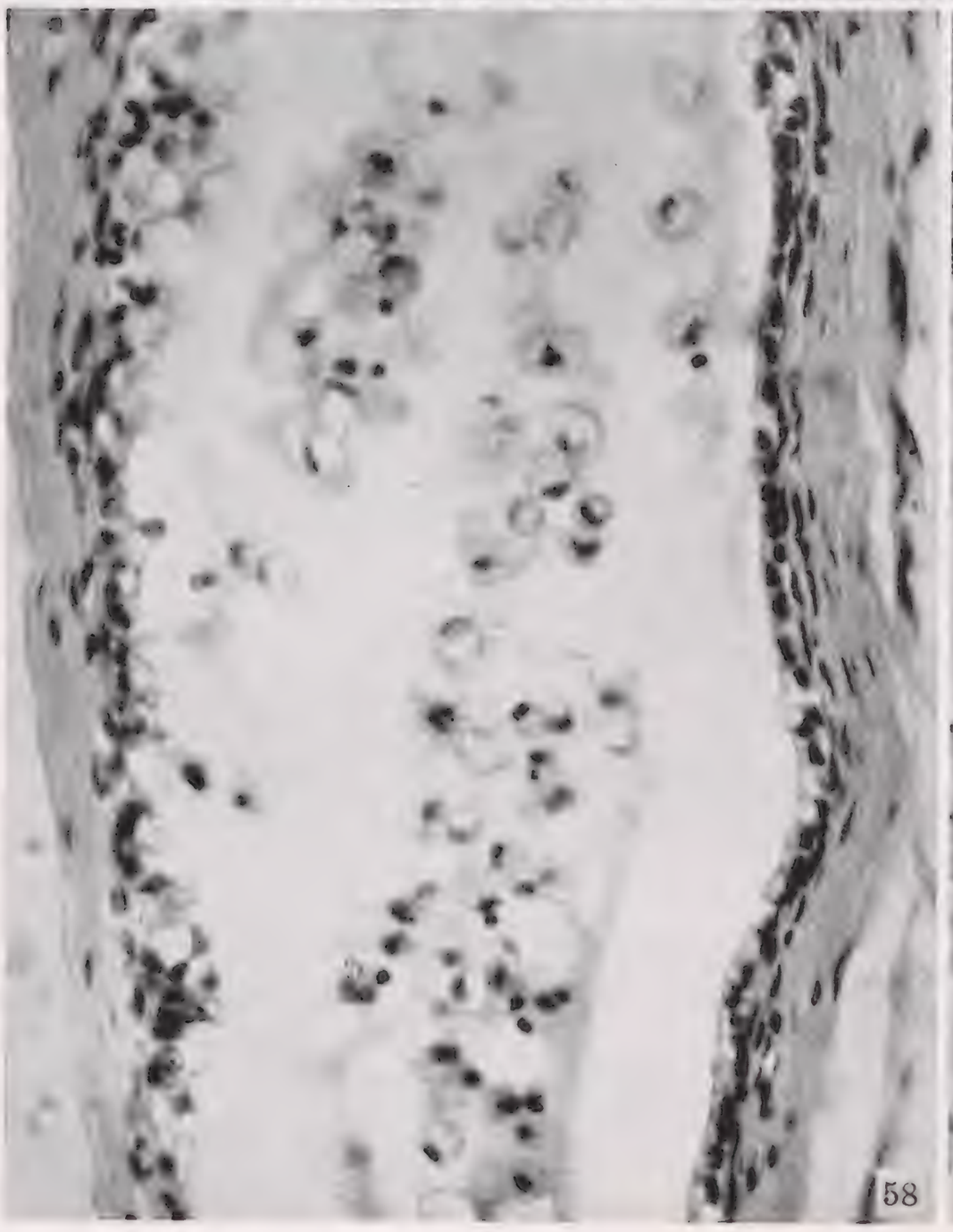

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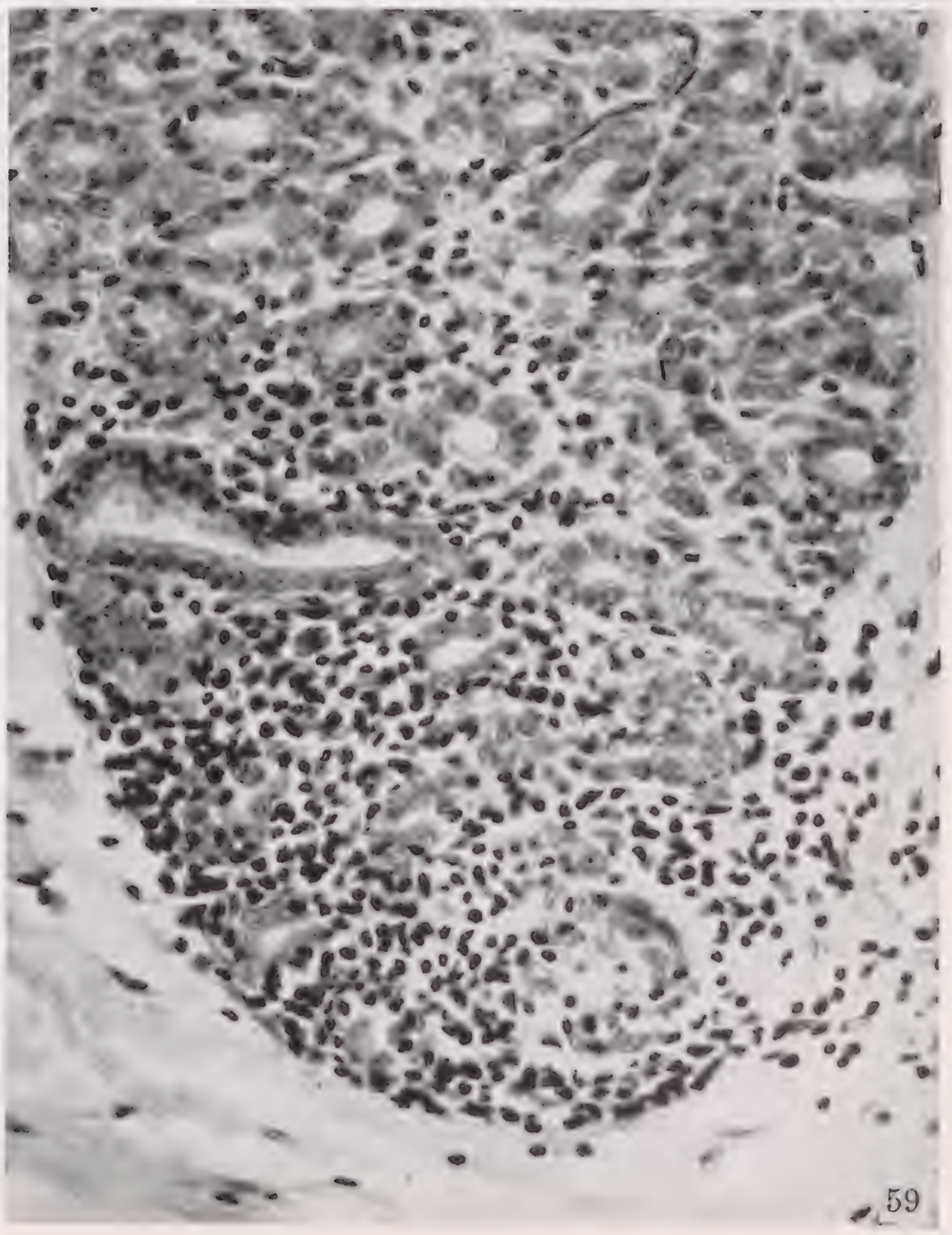

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## Plate 11

Figs. 60–67. Monkey no. 592. Biopsy specimens of mammary gland at various intervals after castration.

Figs. 60–63. Gross mounts; alum cochineal; ×12.

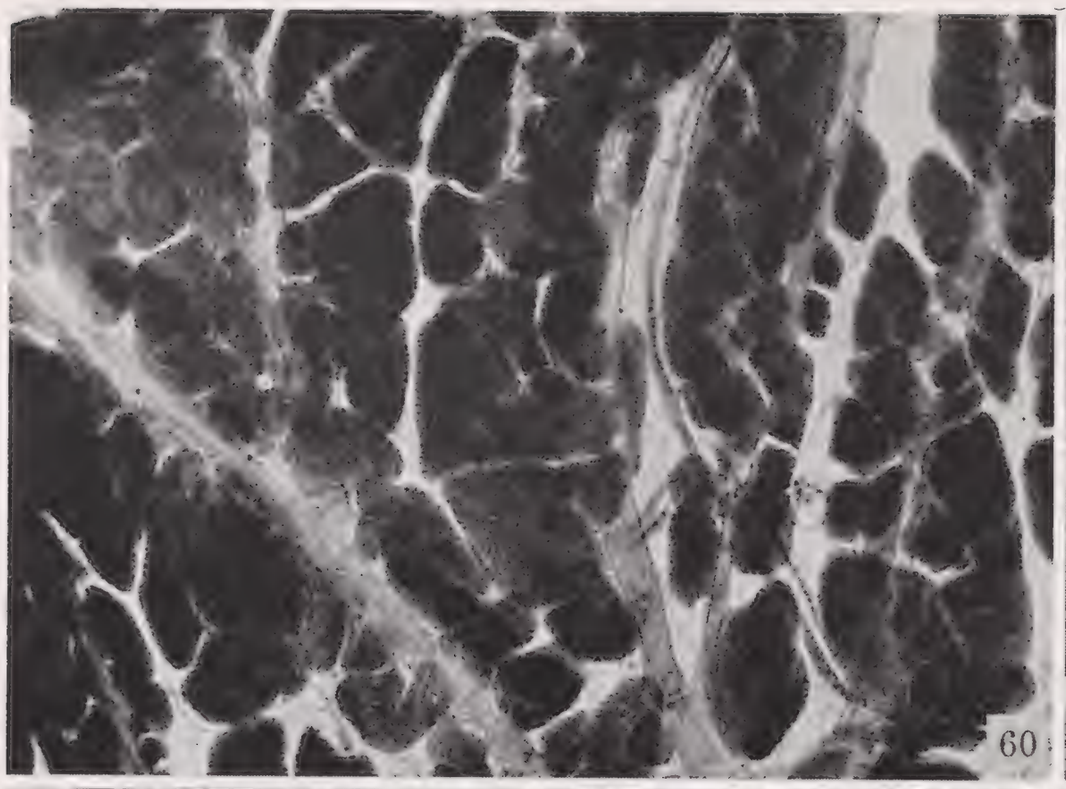
Figs. 64–67. Histologic sections; haematoxylin and eosin; ×60.

Figs. 60, 64. At time of castration.

Figs. 61, 65. 10 days after castration.

Figs. 62, 66. 30 days after castration.

Figs. 63, 67. 58 days after castration.



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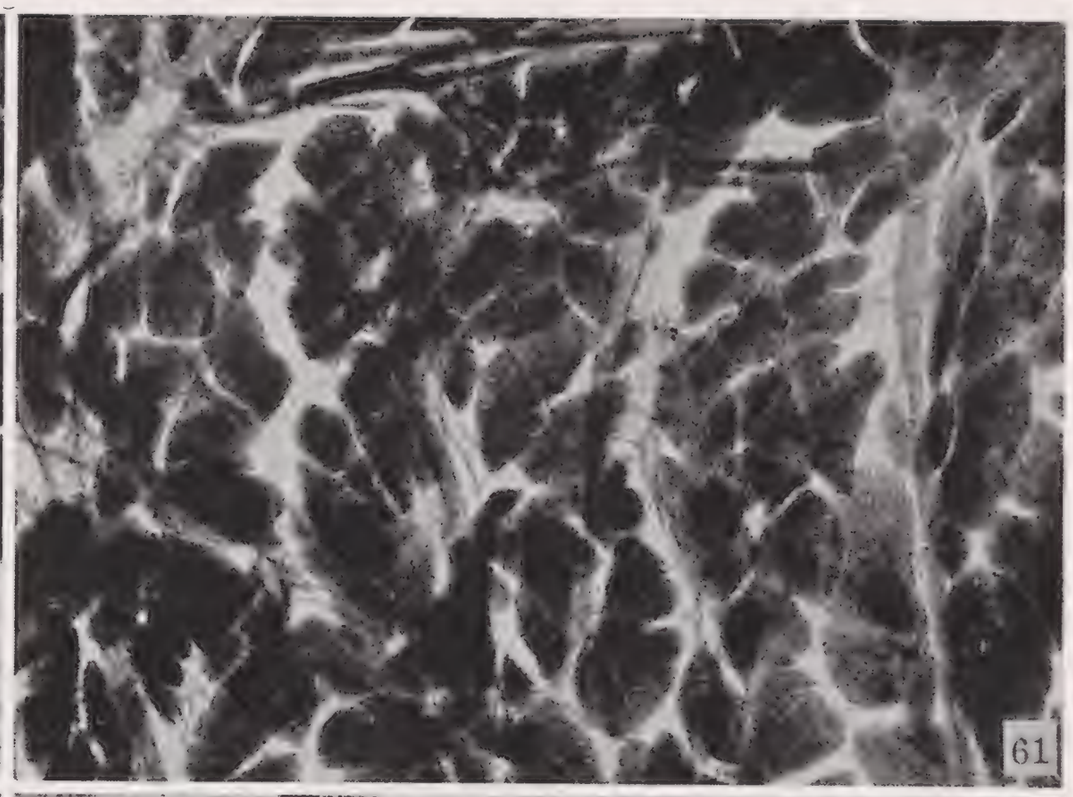

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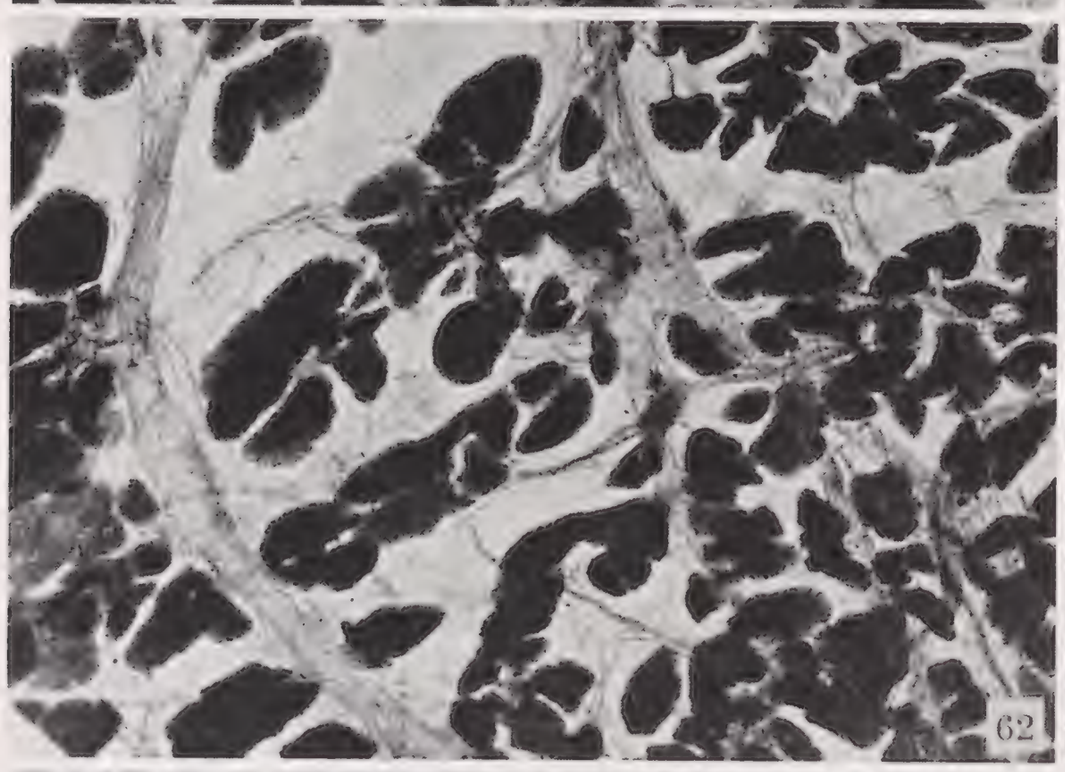

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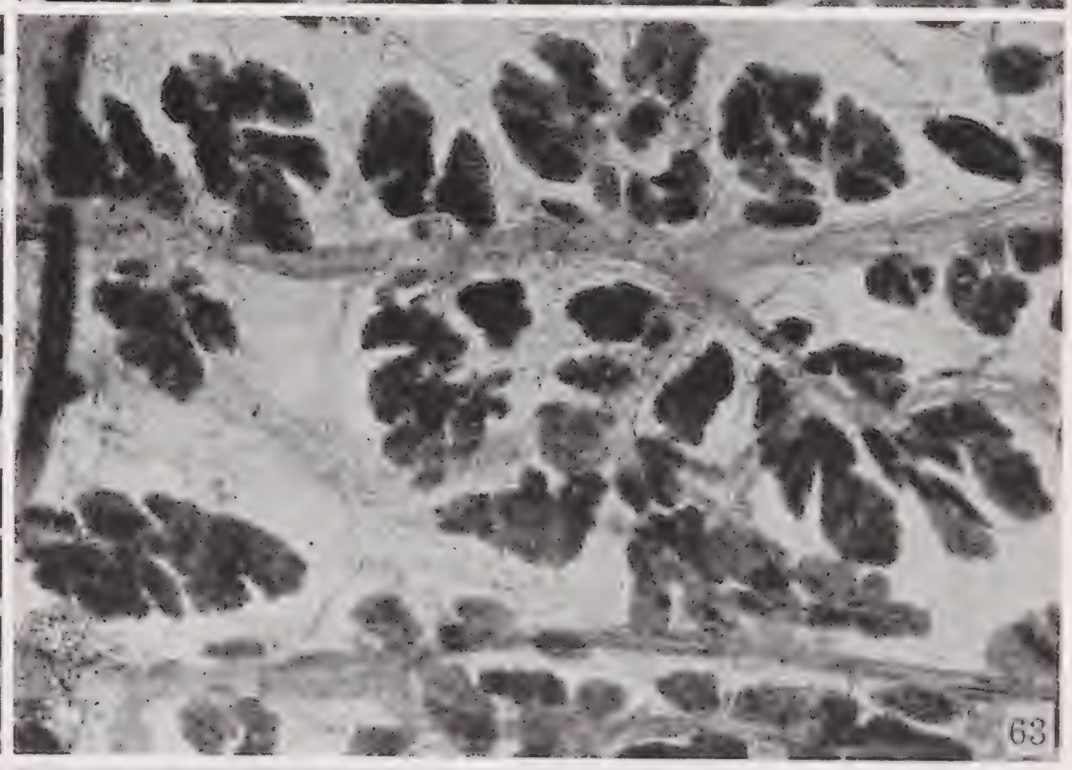

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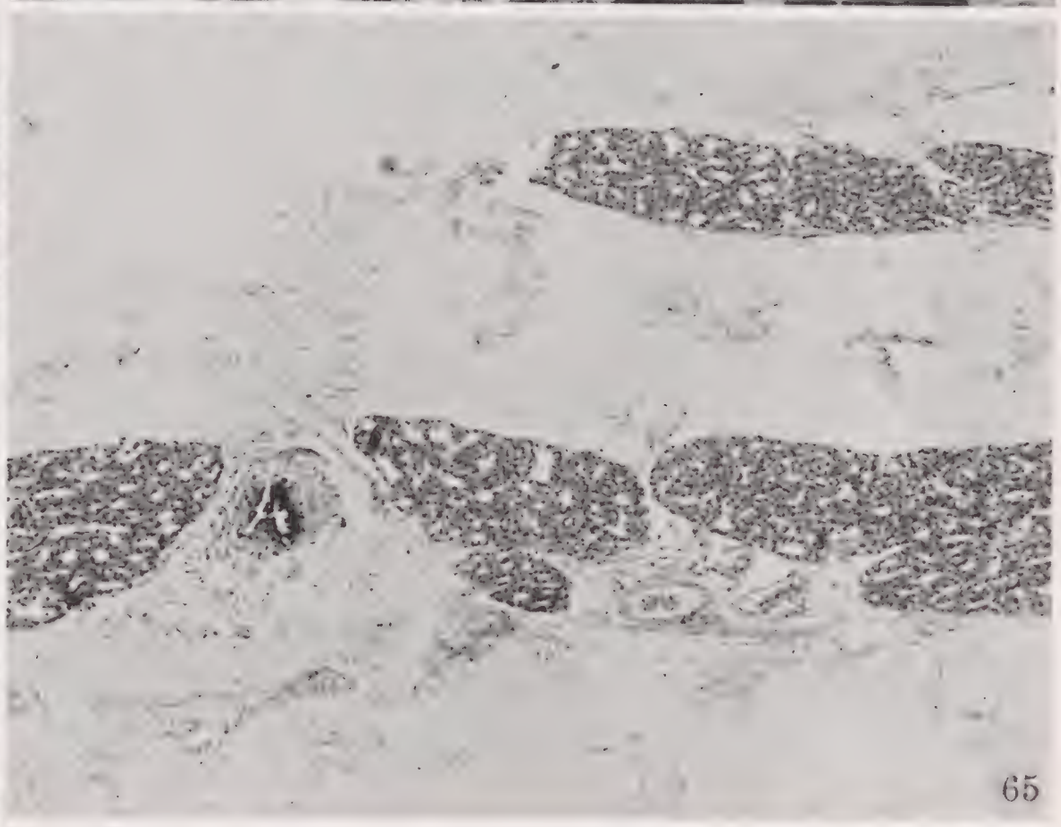

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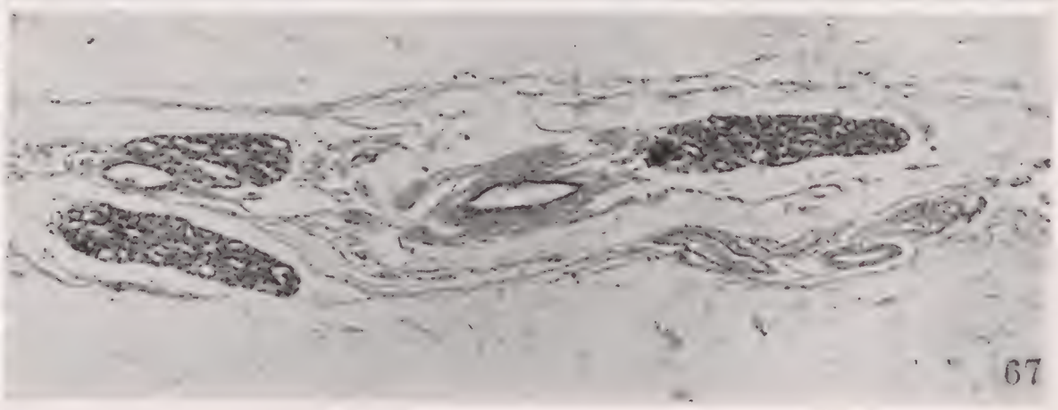

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## Plate 12

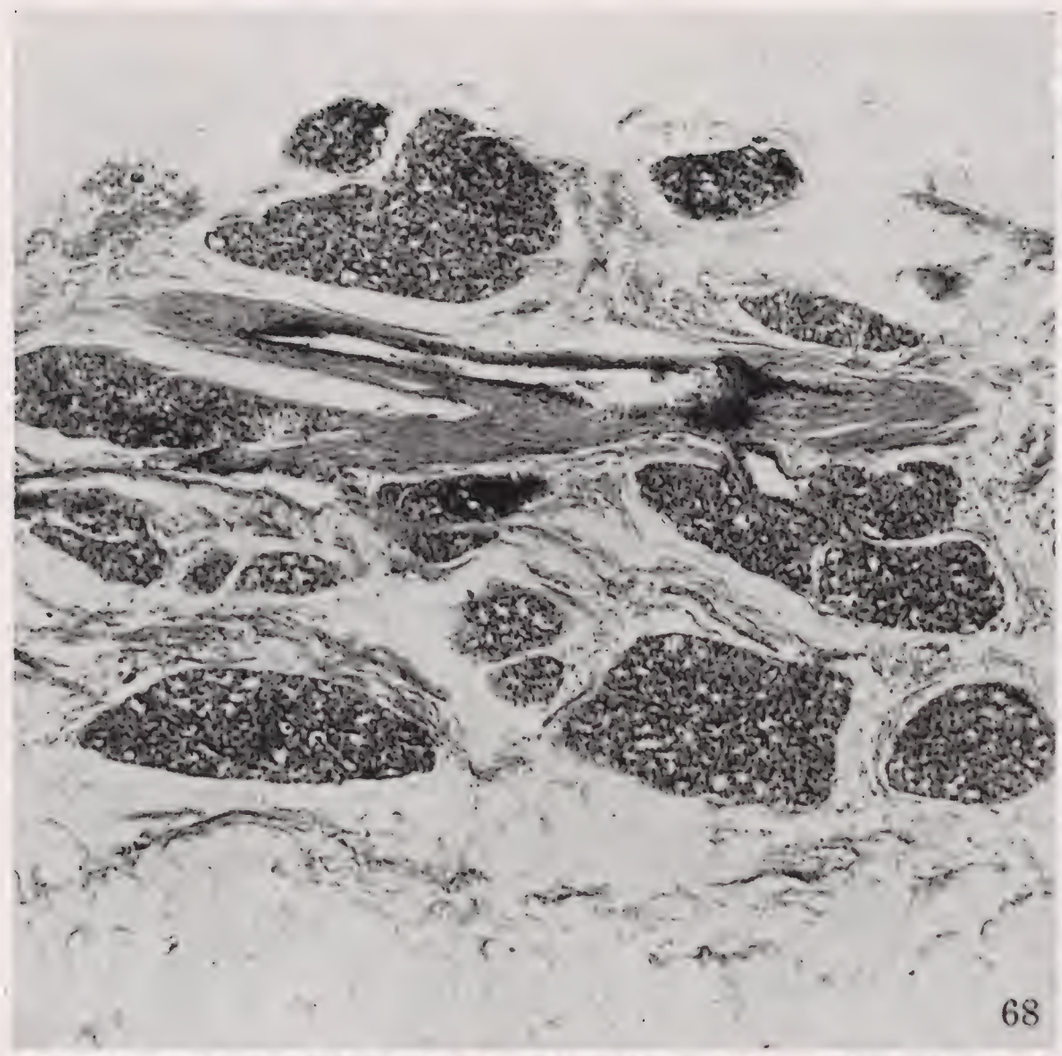
Figs. 68, 69. Monkey no. 623. Biopsy specimens of mammary gland, showing effects of castration on connective tissue. Haematoxylin and eosin; ×50.

Fig. 68. At time of castration.

Fig. 69. 50 days later.

Fig. 70. Monkey no. 687. Mammary gland 76 days after castration, showing nodule in contrast with atrophic lobules. Gross mount; alum cochineal; ×12.

Fig. 71. Monkey no. 57. Mammary gland of amenorrheic animal with ovarian cysts, showing nodules similar to those which occurred after castration. Gross mount; alum cochineal: ×4.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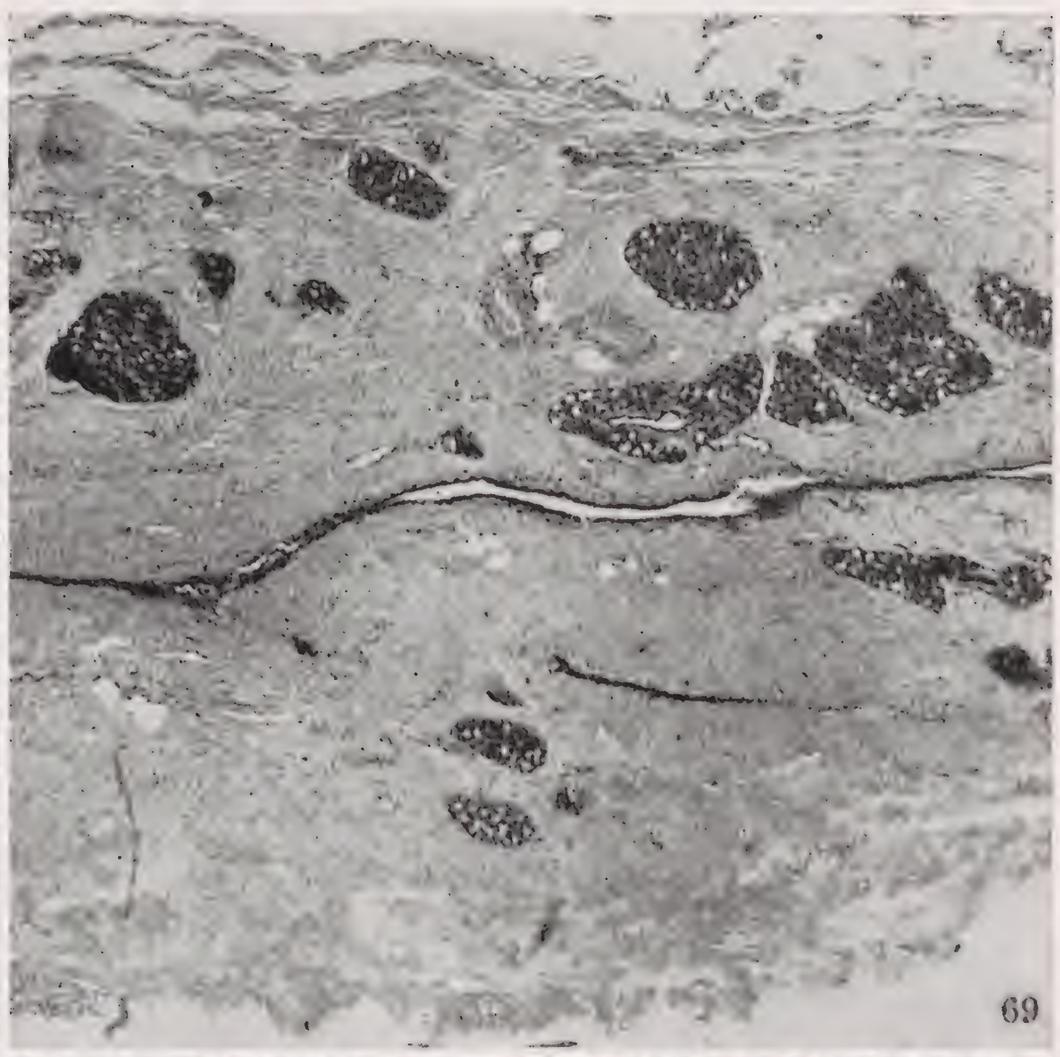
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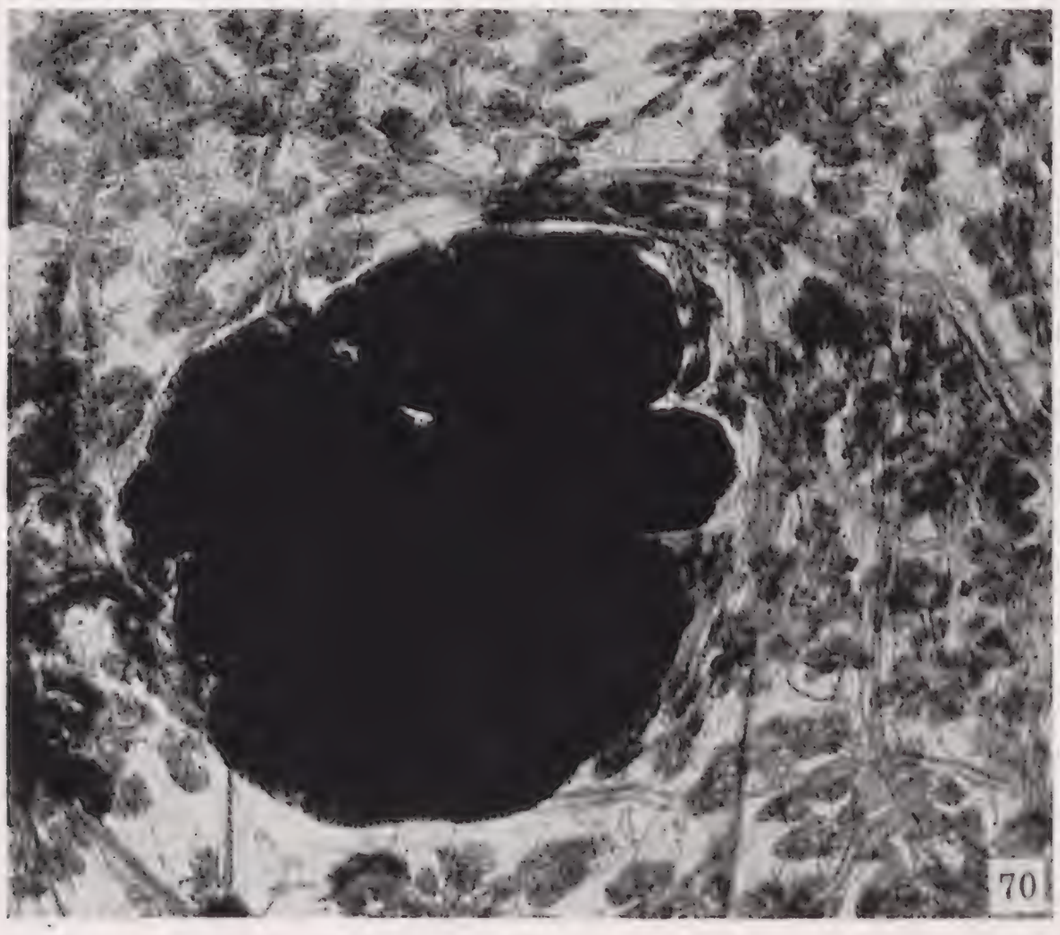
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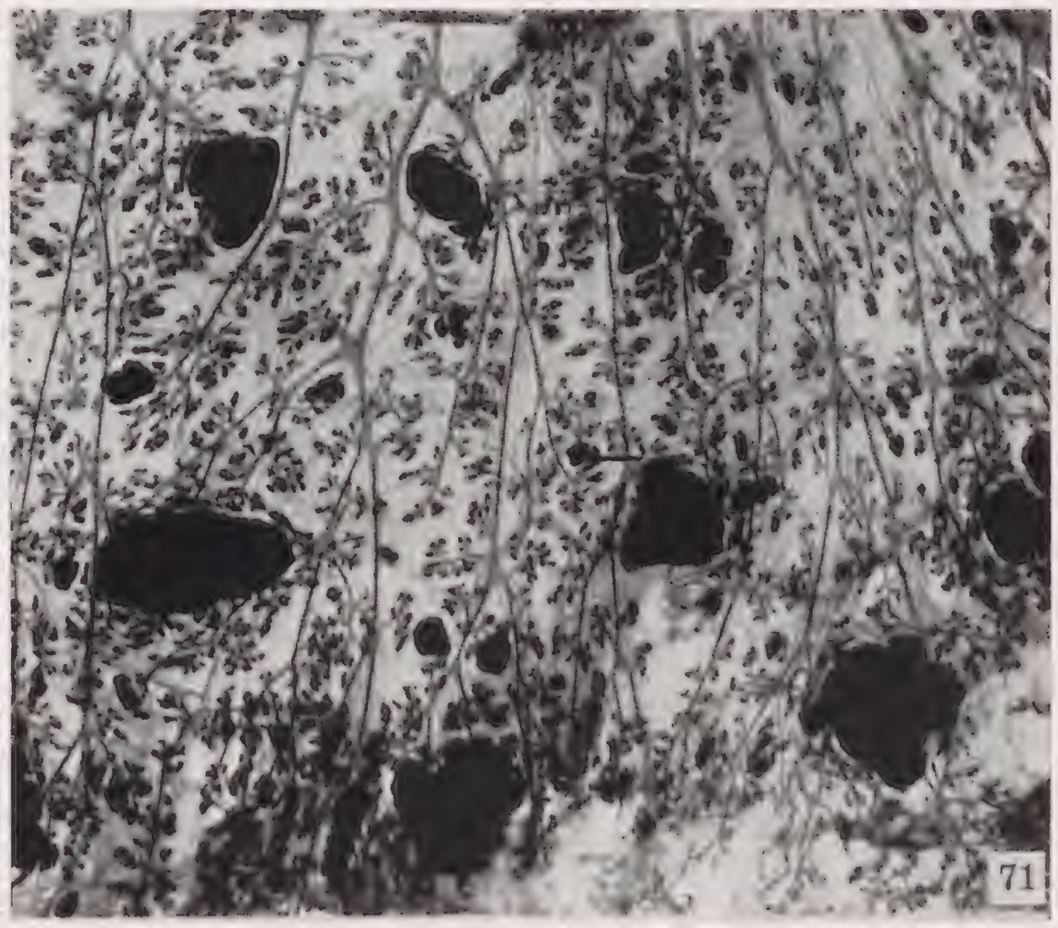
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## Plate 13

Figs. 72–75. Monkey no. 693, adult female. Biopsy specimens of mammary gland before and after treatment with 50 r.u. daily of estradiol benzoate for 30 days.

Figs. 72, 73. Gross mounts; alum cochineal; ×12.

Figs. 74, 75. Histologic sections; haematoxylin and eosin; ×50.

Figs. 72, 74. Before treatment.

Figs. 73, 75. After treatment.

Fig. 76. Monkey no. G26, immature female. Section of mammary gland 12 days after beginning intensive estrogenic treatment consisting in subcutaneous implantation of 95 mg. of estradiol, followed by five 3-mg. pellets weekly and injection of 30,000 I.U. of estradiol every other day. Hyperplastic epithelium of ducts has almost occluded duct lumina. Haematoxylin and eosin; ×100.

Figs. 77, 78. Monkey no. G25, castrated female. Biopsy specimens of mammary gland during estrogenic treatment consisting in injection of 10,000 I.U. of Amniotin every other day and subcutaneous implantation of two 3-mg. pellets of estrone each week. Haematoxylin and eosin; ×100.

Fig. 77. After 36 days' treatment. Ducts are hyperplastic.

Fig. 78. After 17 months' treatment. The lobules of alveoli are completely developed, and the duct epithelium has regressed to one layer of cells.

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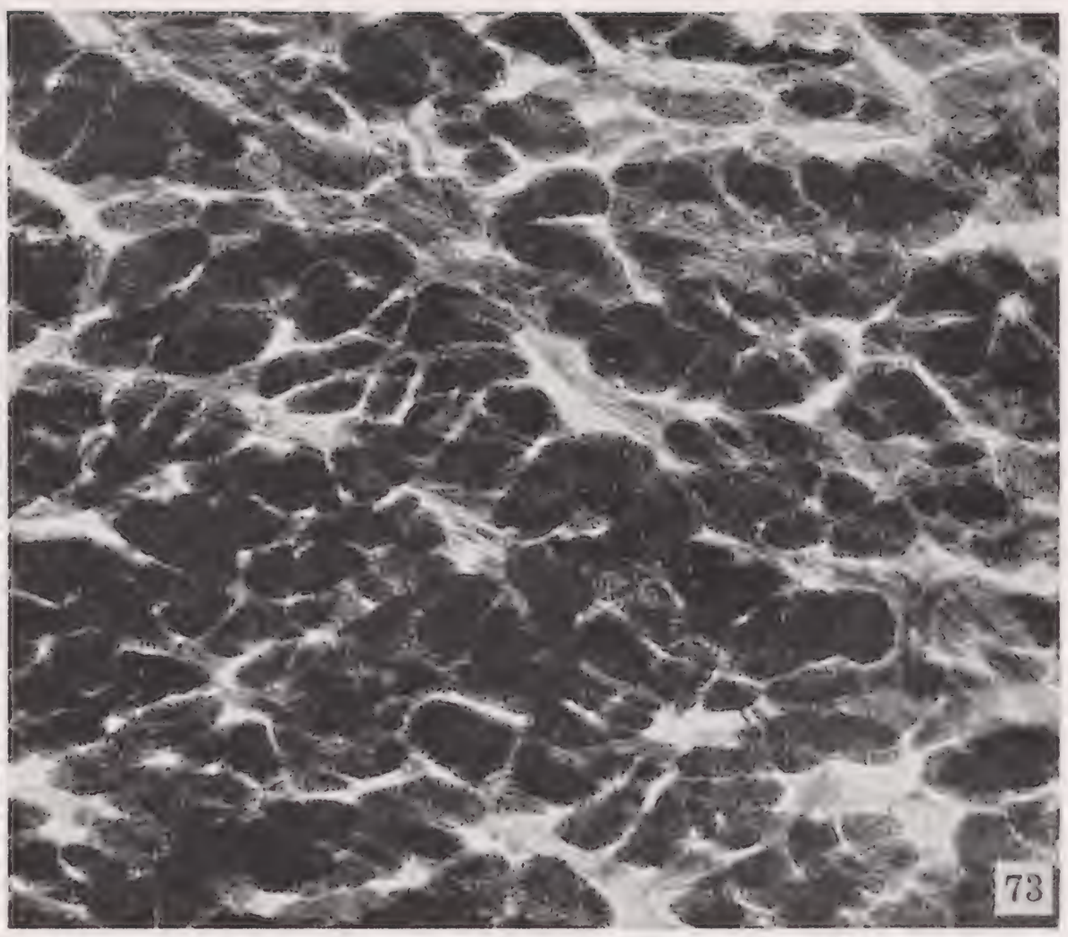
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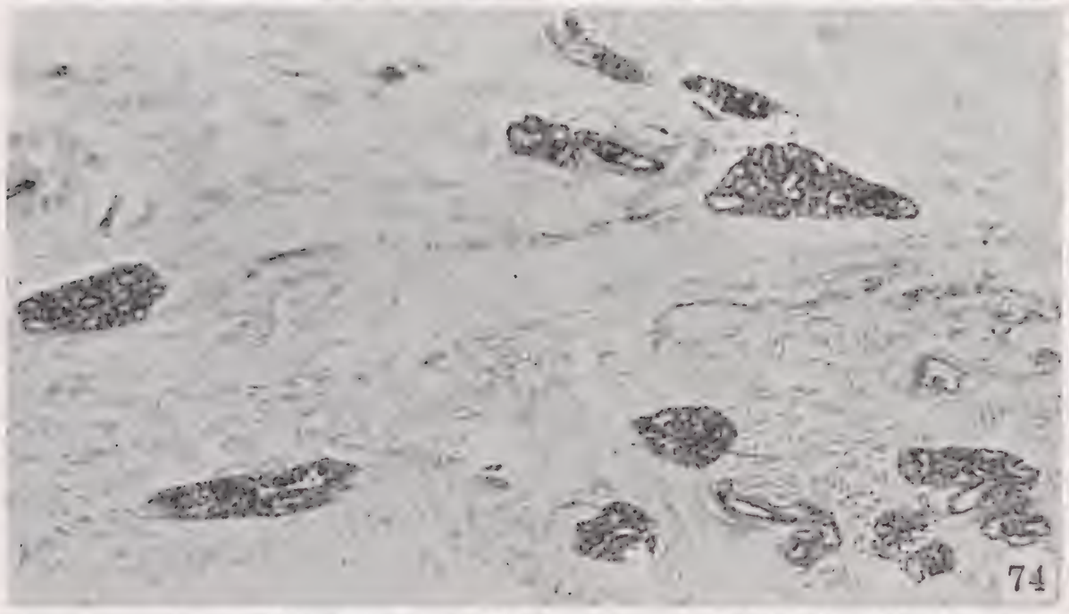
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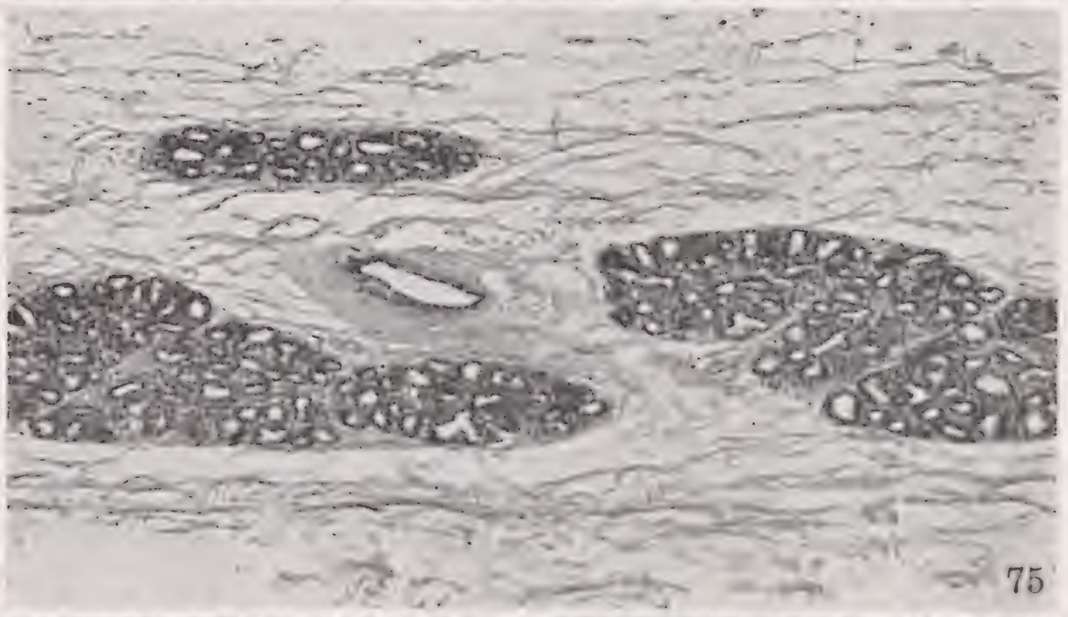
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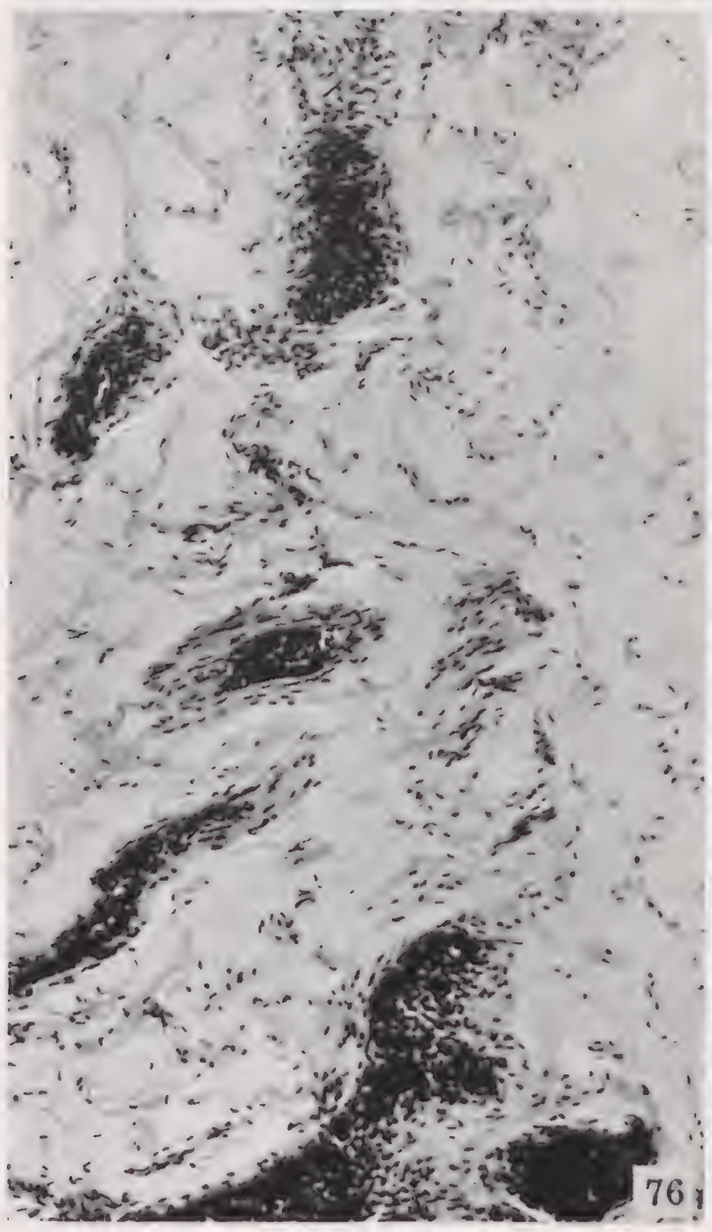
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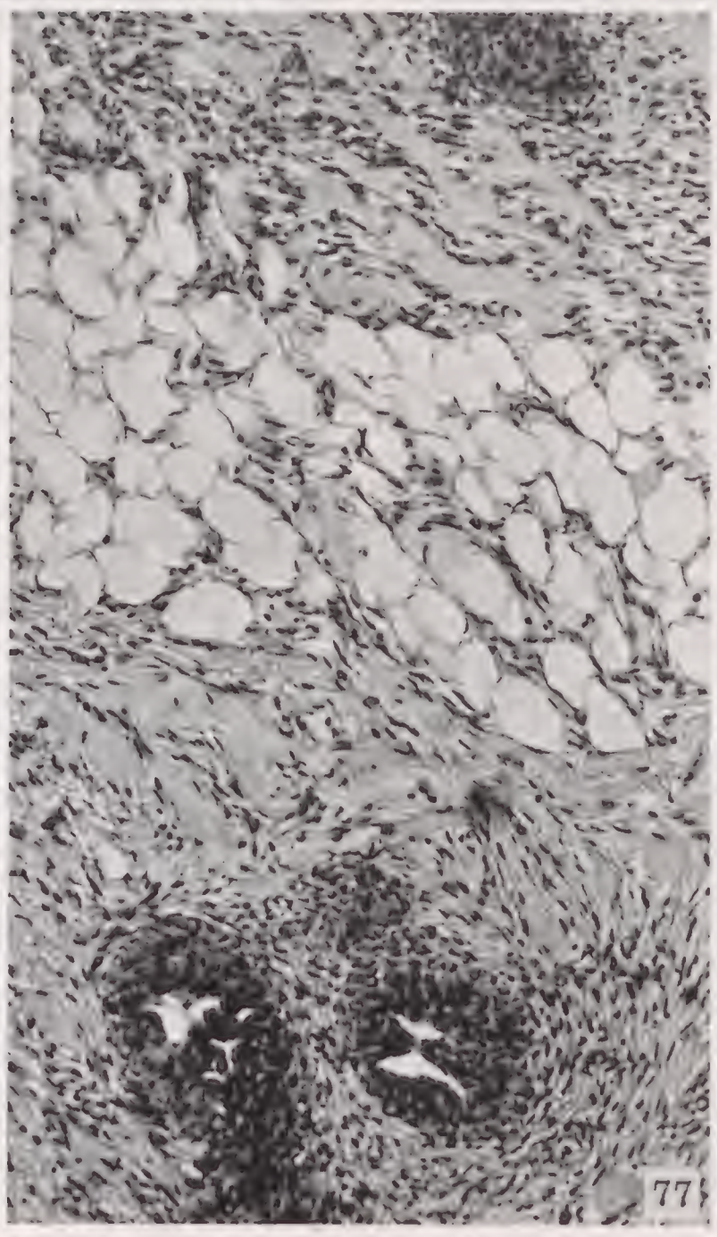
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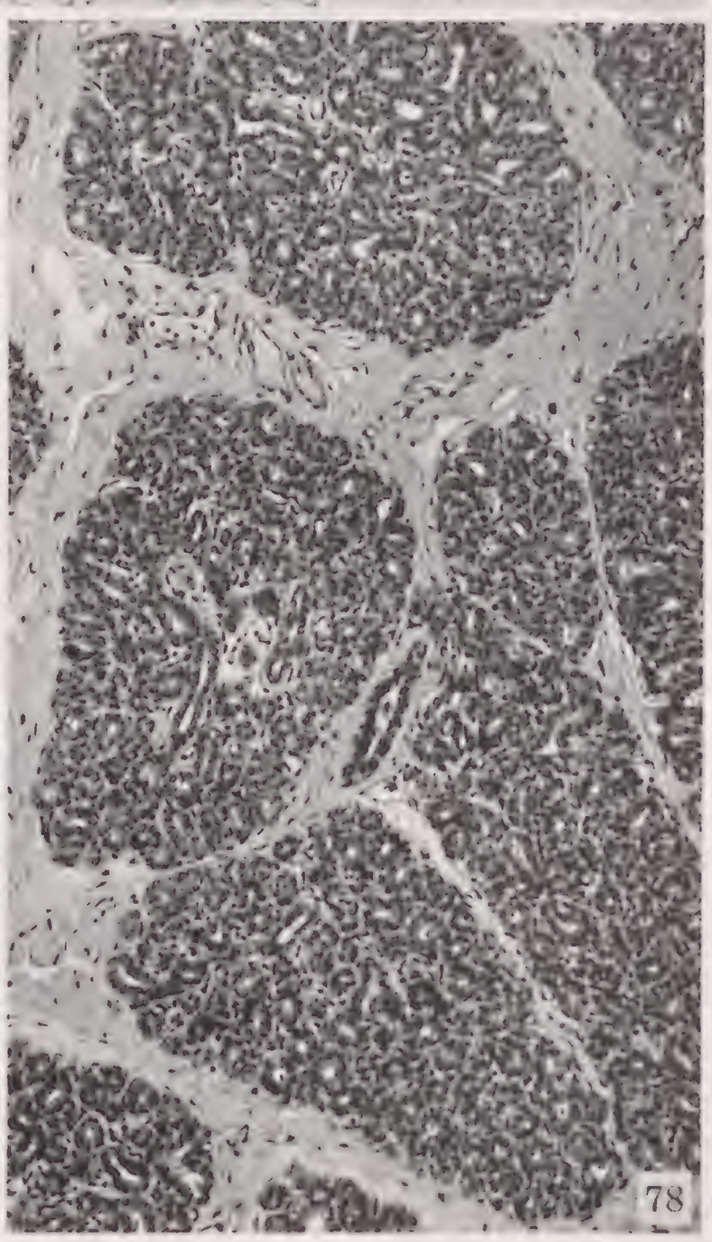

78

## Plate 14

Figs. 79, 80. Monkey no. 560, prepubertal female. Biopsy specimens of mammary gland before and after treatment with 50,000 I.U. of estradiol daily for 70 days. Gross mounts; alum cochineal; ×25.

Fig. 79. Before treatment. Gland consists of branching ducts with few end buds.

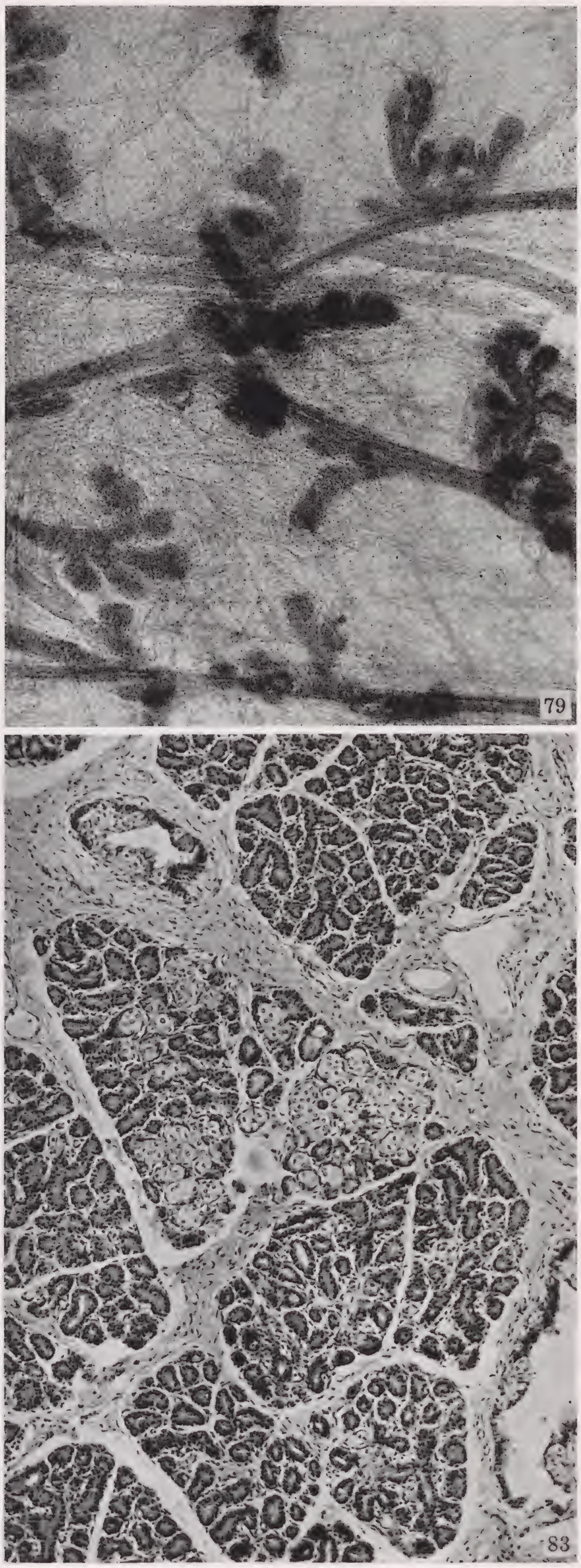
Fig. 80. After treatment. Lobules of alveoli are completely developed.

Figs. 81, 82. Monkey no. G23, castrated prepubertal female. Mammary glands before and after estrogenic treatment, same as no. G25 (pl. 13), for 85 days. Gross mounts; alum cochineal; ×3.

Fig. 81. Before treatment. Gland consists of bare duct tree.

Fig. 82. After treatment. Lobules of alveoli are well developed, but gland has not increased in area.

Fig. 83. Monkey no. G24, castrated female. Biopsy specimen of mammary gland after 14 months of estrogenic treatment, same as no. G25 (pl. 13). Note contrast between pale epithelium and normal mammary cells in lobules and ducts. Haematoxylin and eosin; ×100. Similar metaplasia occurred in the breasts of one-third of the animals treated with very large amounts of estrogen.



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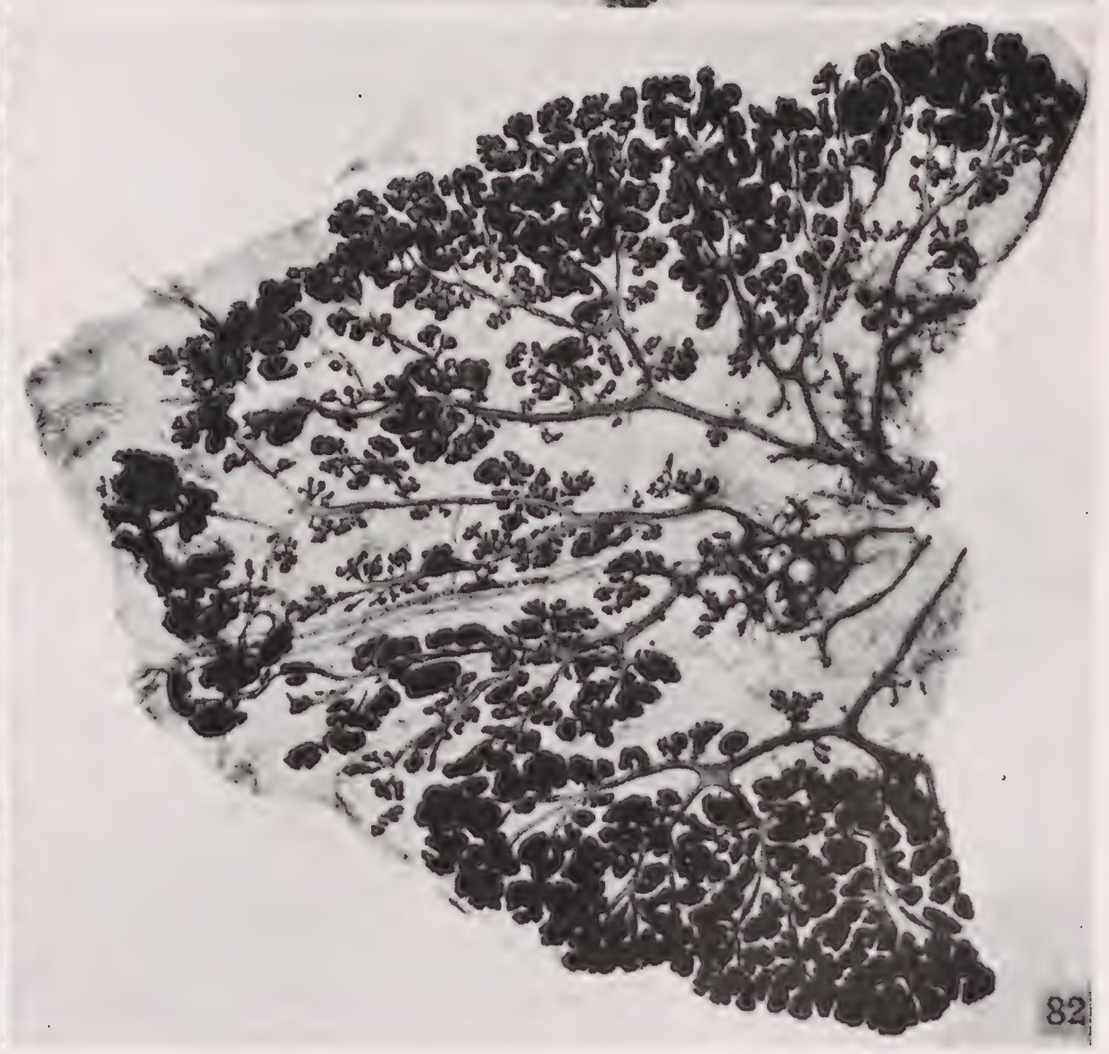

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## Plate 15

Figs. 84, 85. Monkey no. 425, castrated female. Mammary gland before and after combined treatment with estrogen and progestin. Haematoxylin and eosin; ×100.

Fig. 84. Before treatment.

Fig. 85. After treatment consisting in 100 r.u. of Amniotin daily for 12 days and 1 cc. (70 gm. equiv.) of progestin daily, beginning on the 8th day, for 18 consecutive days.

Figs. 86, 87. Monkey no. 673, castrated female. Biopsy specimens of mammary gland before and after treatment with progesterone. Gross mounts; alum cochineal; ×12.

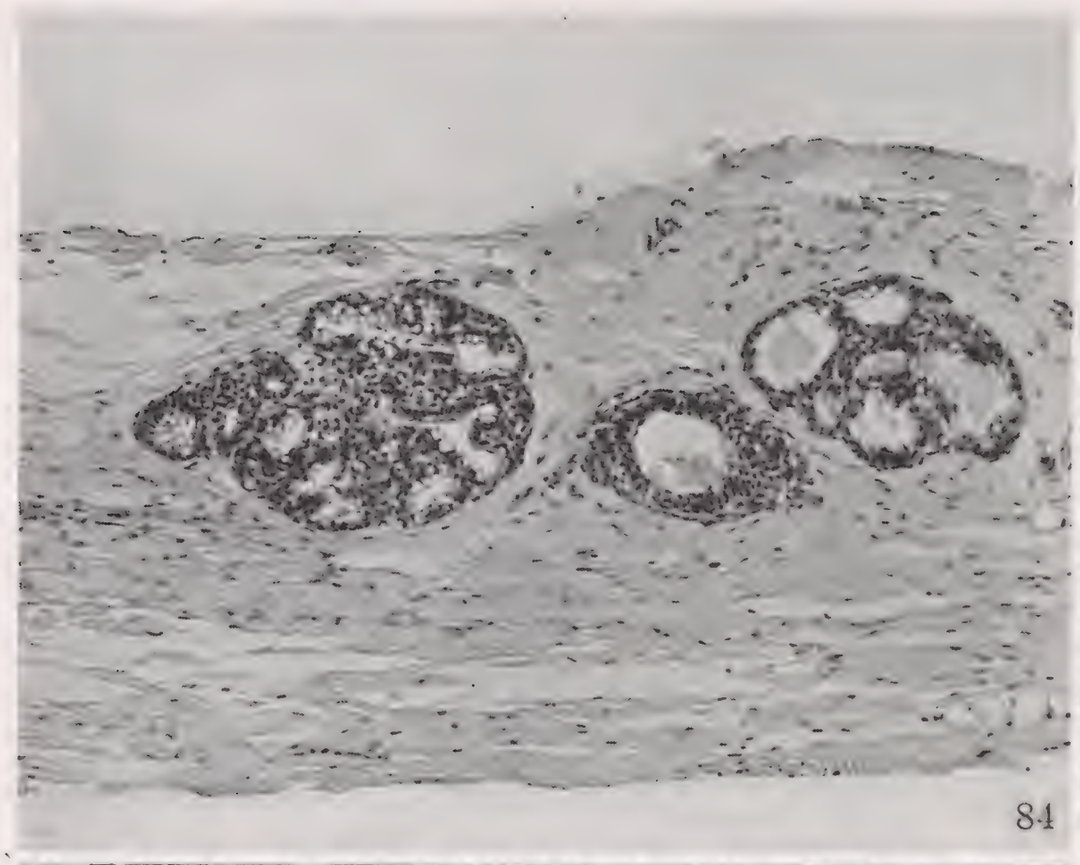
Fig. 86. Before treatment.

Fig. 87. After treatment consisting in 20 mg. of crystalline progesterone daily for 32 days.

Figs. 88, 89. Monkey no. 623, castrated female. Biopsy specimens of mammary gland before and after treatment with progesterone. Haematoxylin and eosin; ×50.

Fig. 88. Before treatment.

Fig. 89. After treatment consisting in 5 mg. of progesterone daily for 32 days. Note change in character of stroma. This effect was also observed in a few of the animals treated with testosterone propionate.



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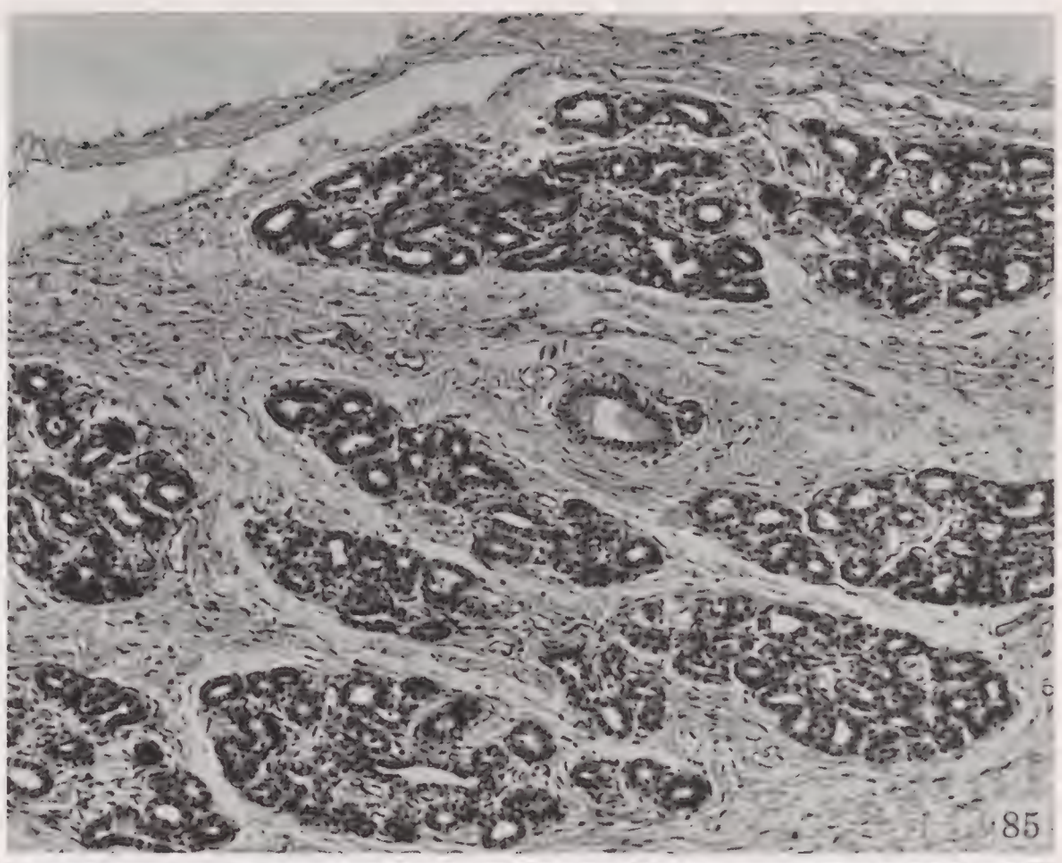

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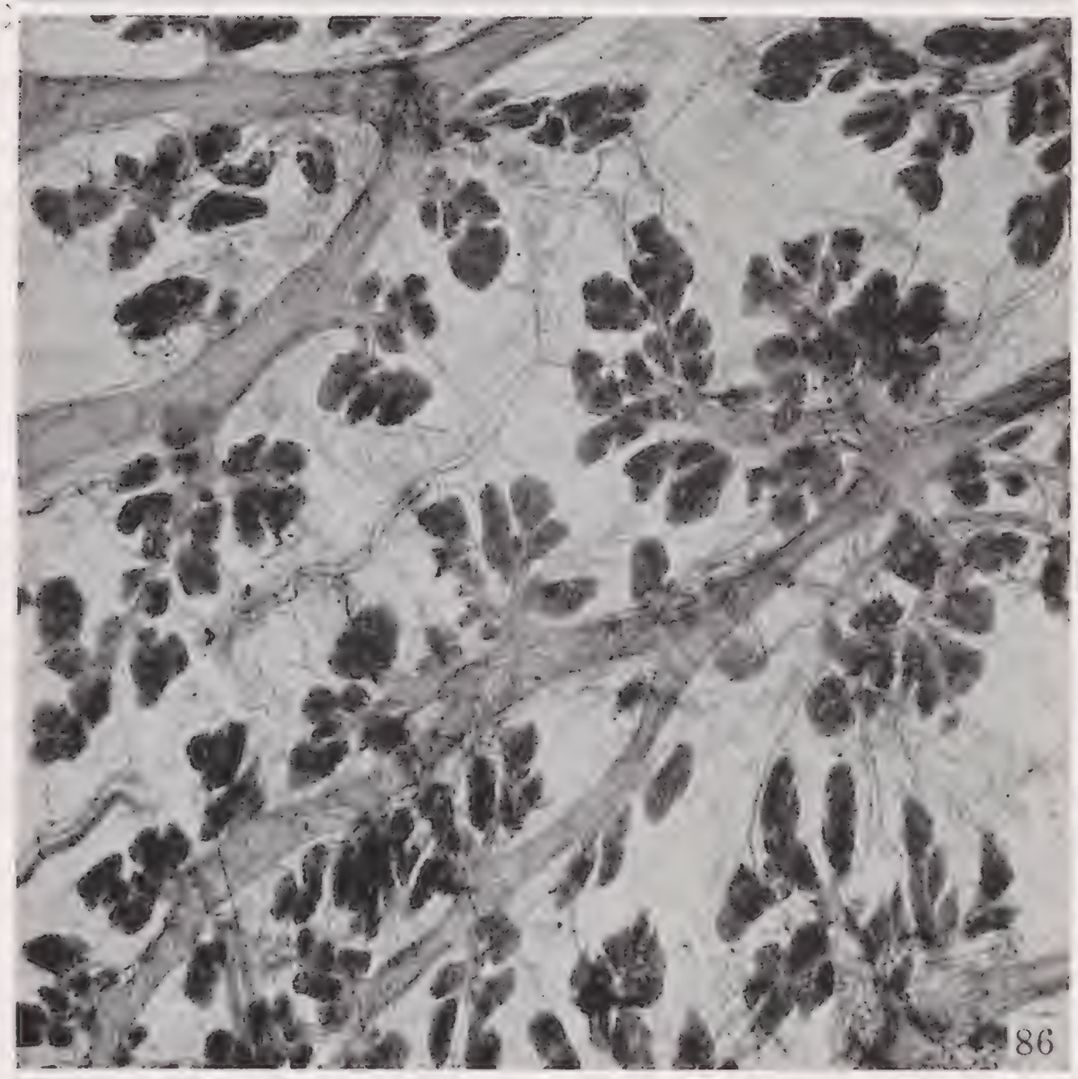

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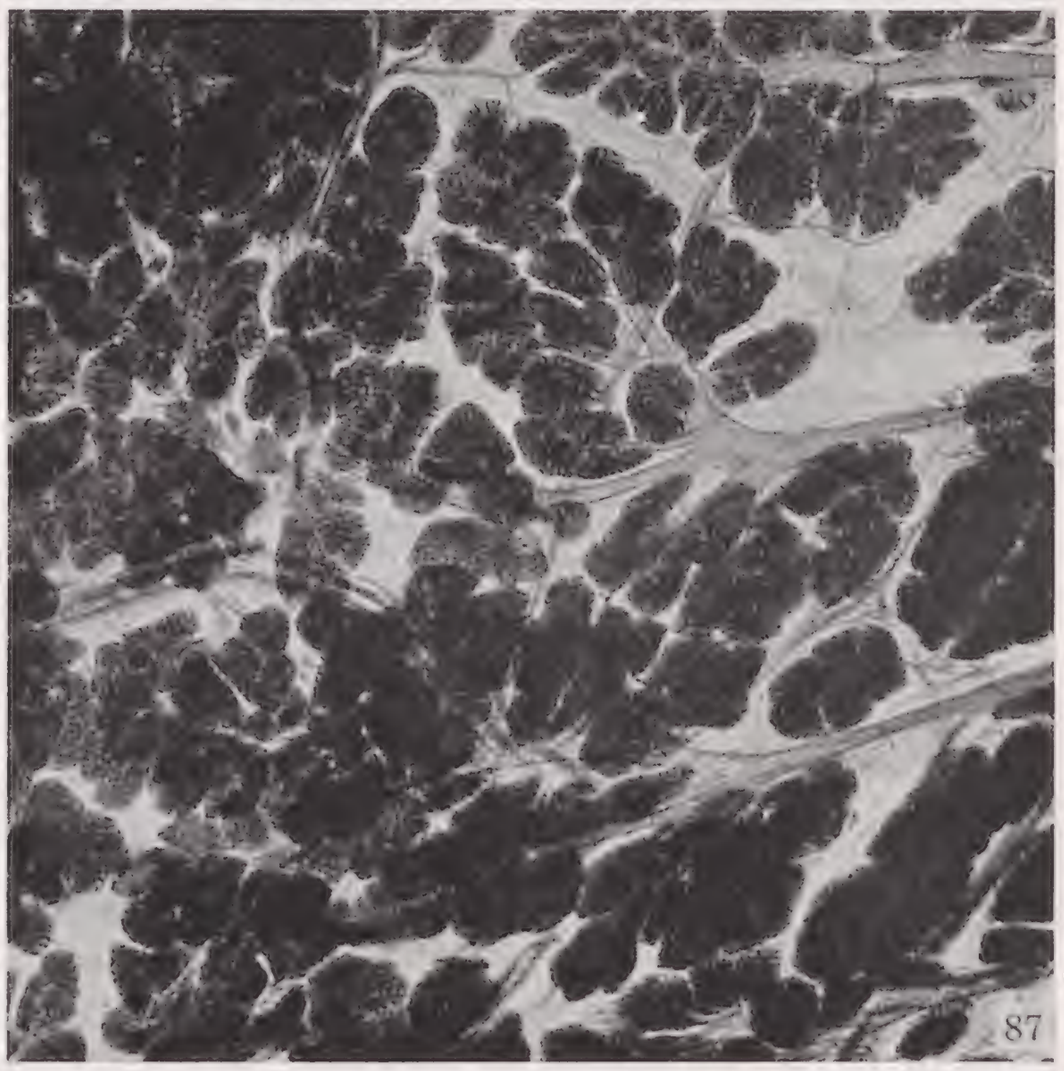

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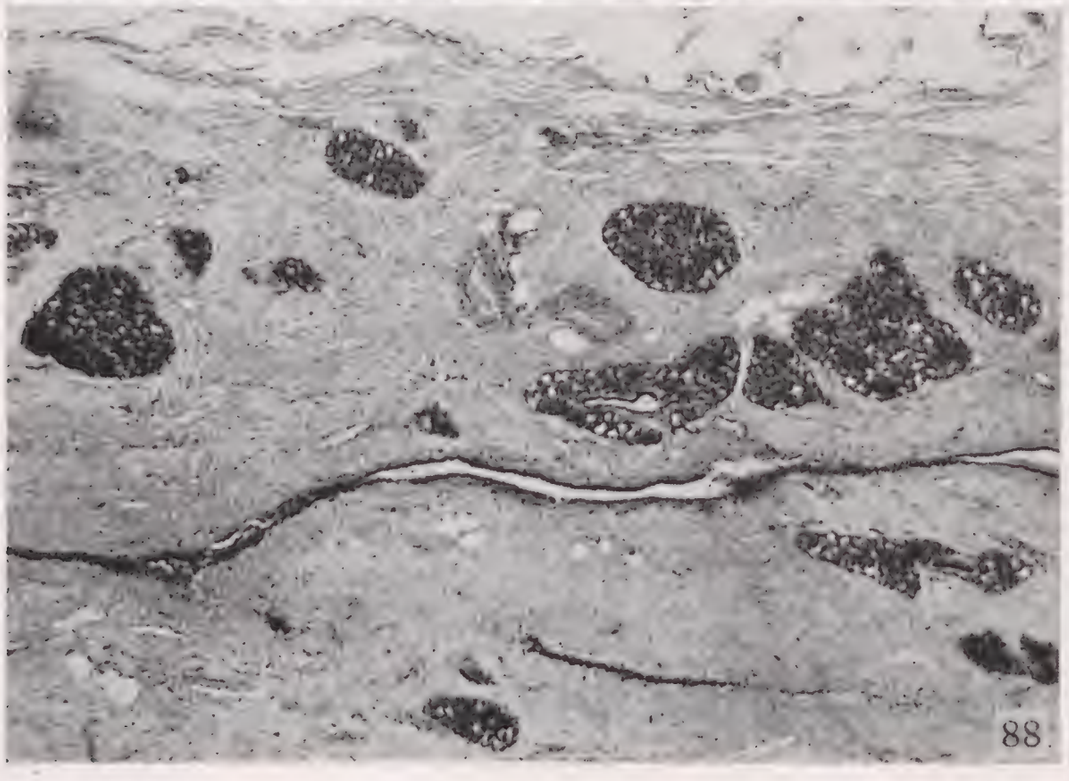

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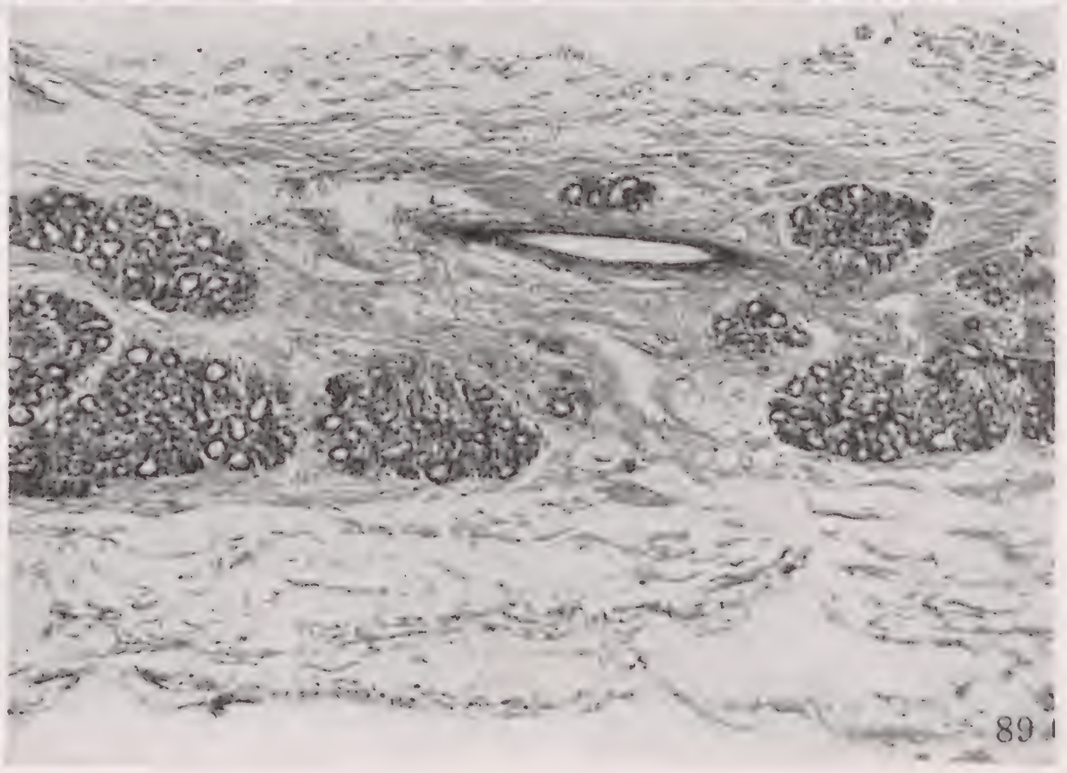

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## Plate 16

Figs. 90, 91. Monkey no. 699, nonovulating, amenorrheic female. Biopsy specimens of mammary gland before and after treatment with gonadotrophic hormones. Gross mounts; alum cochineal; ×12.

Fig. 90. Before treatment.

Fig. 91. After treatment for 35 days with a mixture of FSH and Follutein, followed by 141 units of Gonadogen. Slight development of mammary gland.

Figs. 92, 93. Monkey no. 673, nonovulating, amenorrheic female. Biopsy specimens of mammary gland before and after treatment with gonadotrophic hormones. Gross mounts; alum cochineal; ×12.

Fig. 92. Before treatment.

Fig. 93. After treatment consisting in 2 courses of FSH and Follutein injections and 2 courses of injections with Gonadogen over a period of 4 months. Good development of mammary gland.

Figs. 94–97. Monkey no. 692, castrated female. Biopsy specimens of mammary gland before and after treatment with testosterone propionate, 20 mg. daily for 31 days.

Figs. 94, 95. Gross mounts; alum cochineal; ×12.

Figs. 96, 97. Histologic sections; haematoxylin and eosin; ×50.

Figs. 94, 96. Before treatment. Gland is atrophic, stroma sclerotic.

Figs. 95, 97. After treatment. Lobules have grown, alveoli are patent, and stroma is edematous.

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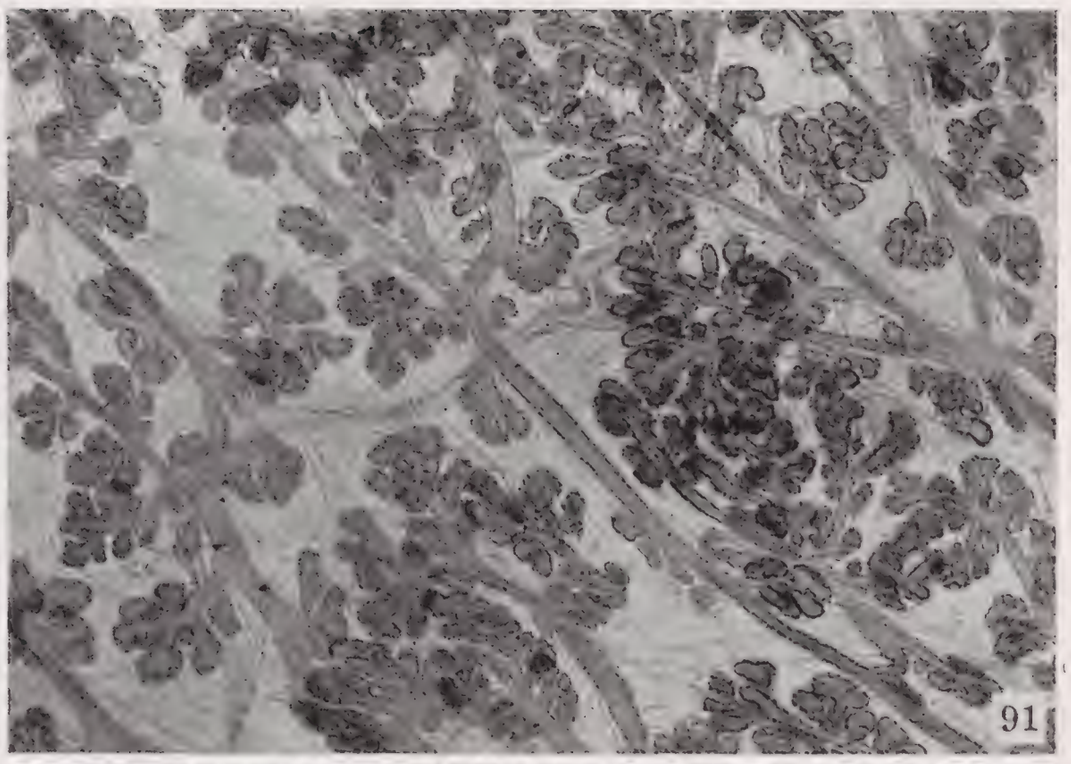
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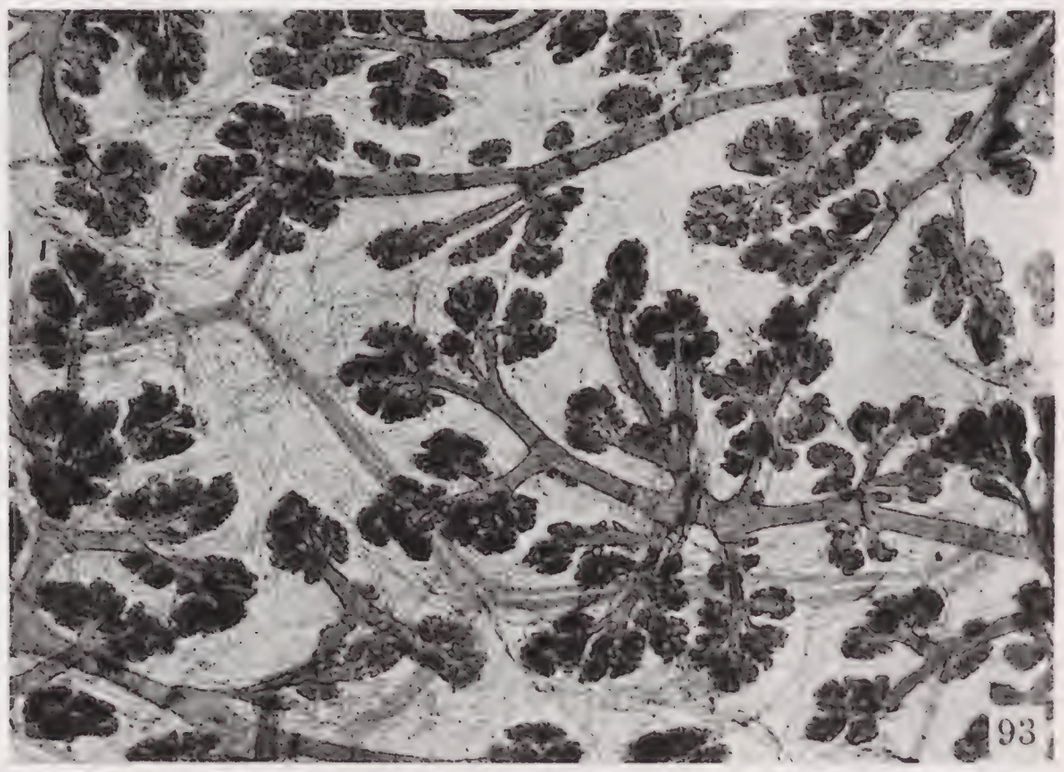
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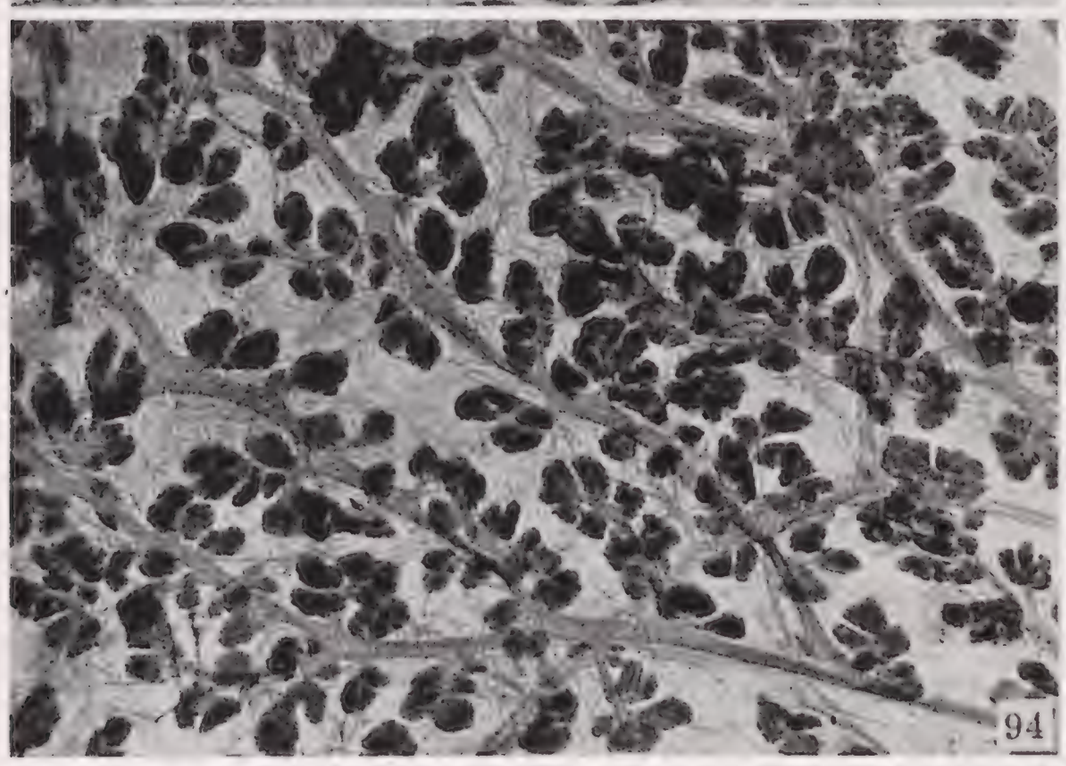
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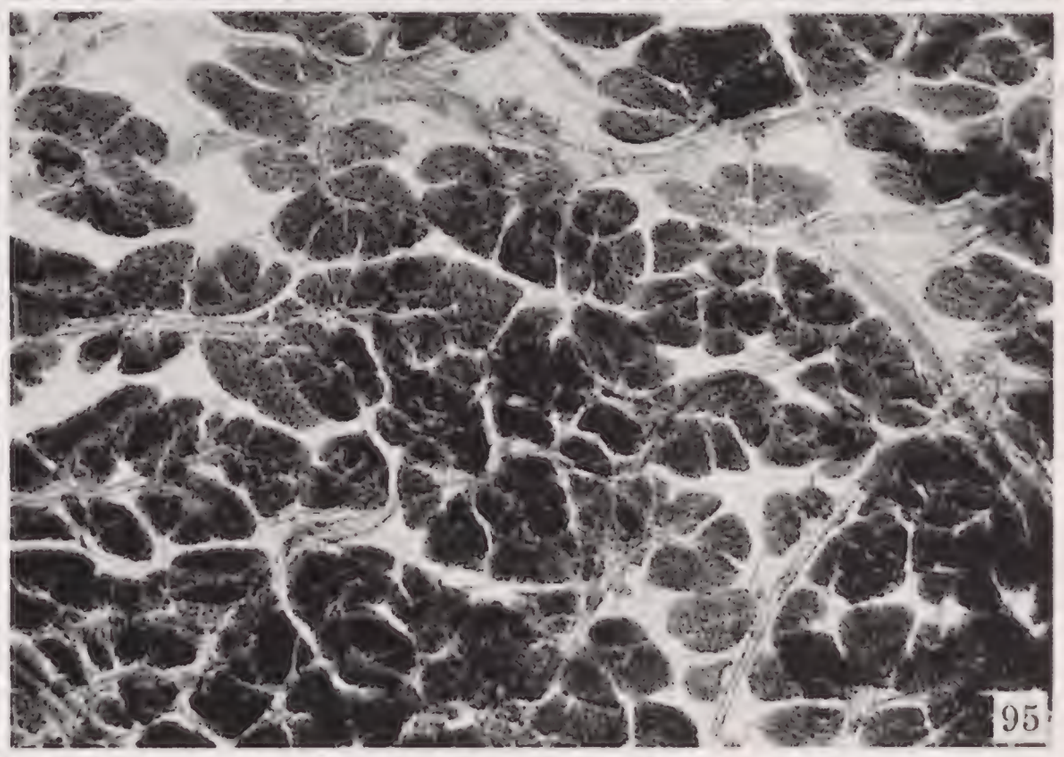
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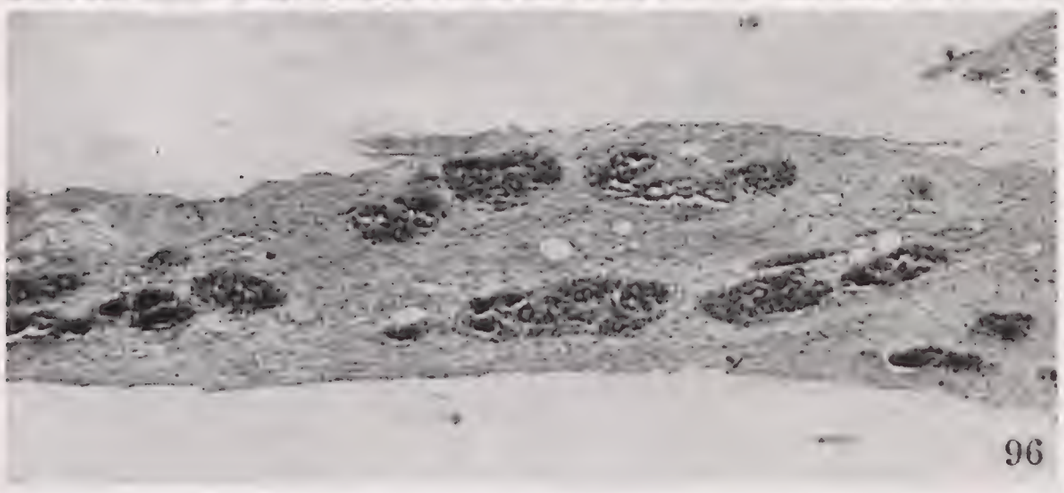
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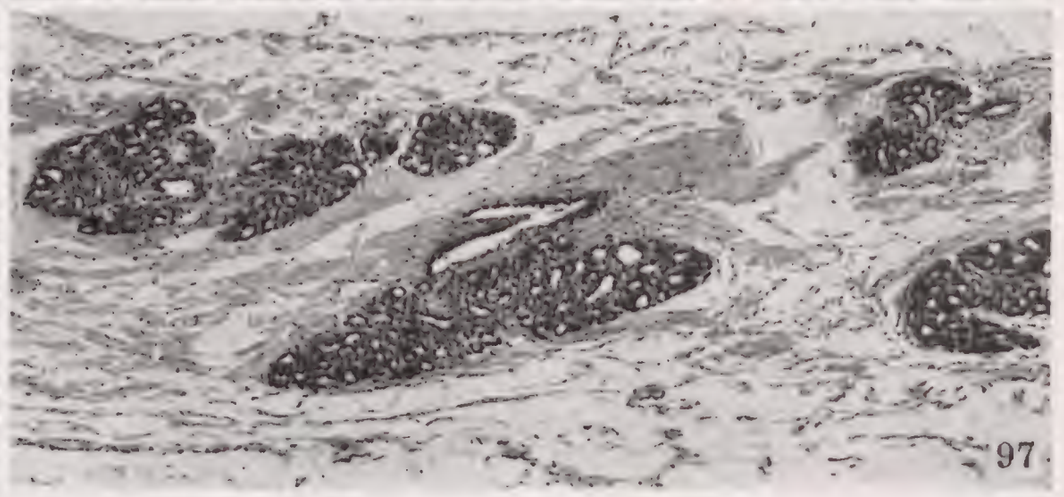
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## Plate 17

Figs. 98, 99. Monkey no. M8, castrated male. Mammary gland before and after treatment with testosterone, 5 mg. daily for 15 days. Gross mounts; alum cochineal; ×3.

Fig. 98. Before treatment. Ducts are slender, few end buds are present.

Fig. 99. After treatment. Moderate dilatation of ducts and slight lobular development have occurred.

Figs. 100, 101. Monkey no. 427, castrated female. Biopsy specimens of mammary gland following treatment with testosterone propionate and 35 days later. Haematoxylin and eosin; ×100.

Fig. 100. After 25 days' treatment with testosterone propionate, 10 mg. daily, showing good lobular development.

Fig. 101. Thirty-five days after cessation of treatment, showing atrophy of lobules.

Figs. 102–106. Monkey no. 602, castrated female. Biopsy specimens of mammary gland before and after treatment with Amniotin, 100 r.u. daily for 12 days, followed by Amniotin, 25 r.u. daily, and testosterone, 50 mg. daily for 15 days.

Figs. 102, 103. Gross mounts; alum cochineal; ×12.

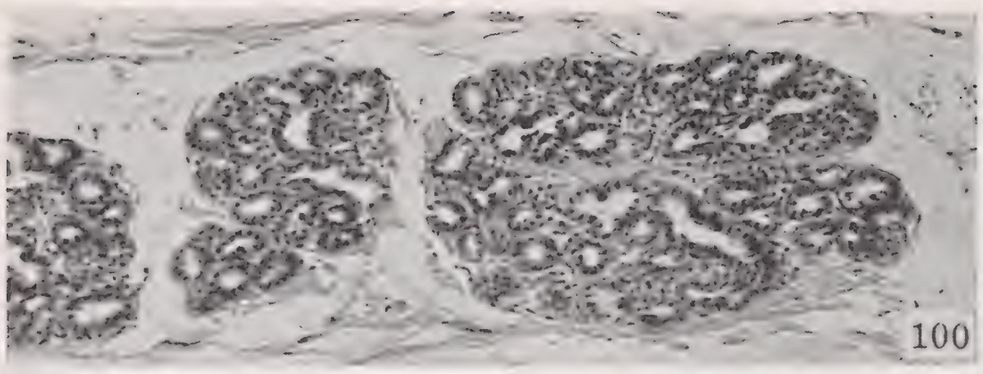
Fig. 104. Histologic section; haematoxylin and eosin; ×100.

Fig. 105. Histologic section; iron haematoxylin; ×100.

Figs. 102, 104. Before treatment. Gland is atrophic.

Figs. 103, 105. After treatment. Striking lobular development has occurred. Ducts and alveoli are dilated and contain clear secretion. Epithelium is puckered in places, producing papillomatous formations.

Fig. 106. Same as figure 105, showing epithelial proliferation. ×250.



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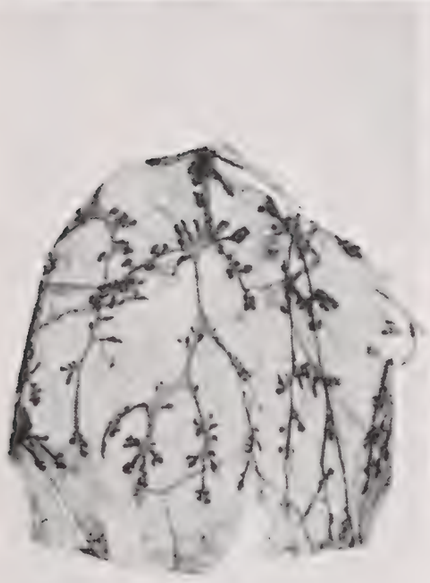

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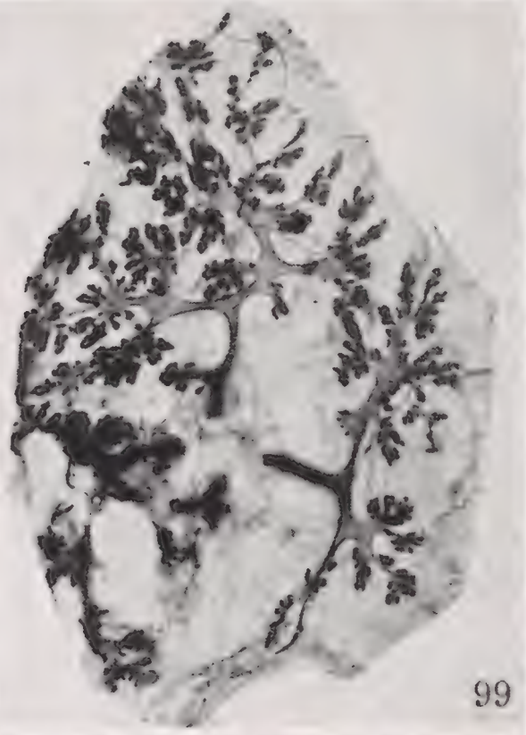

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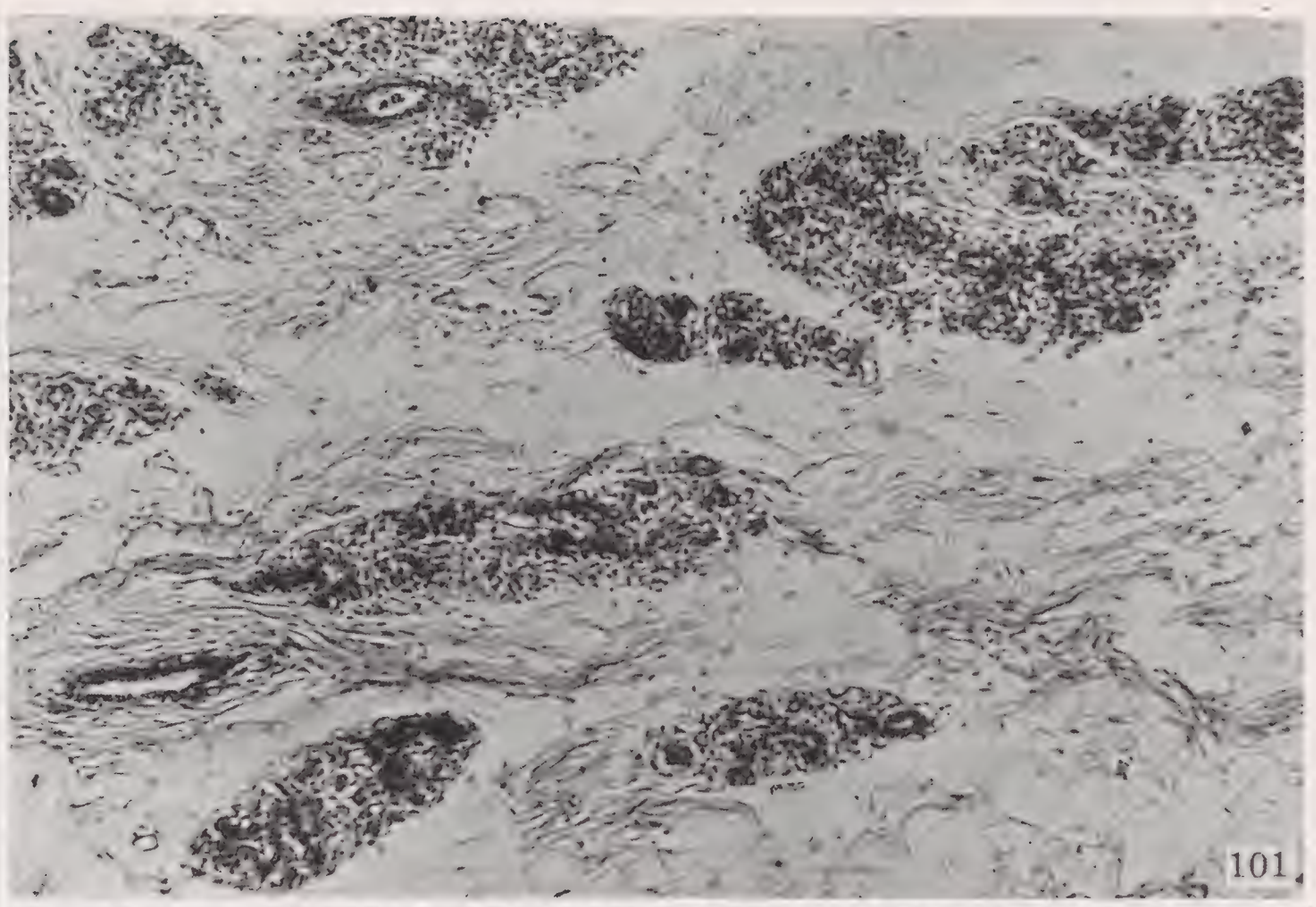

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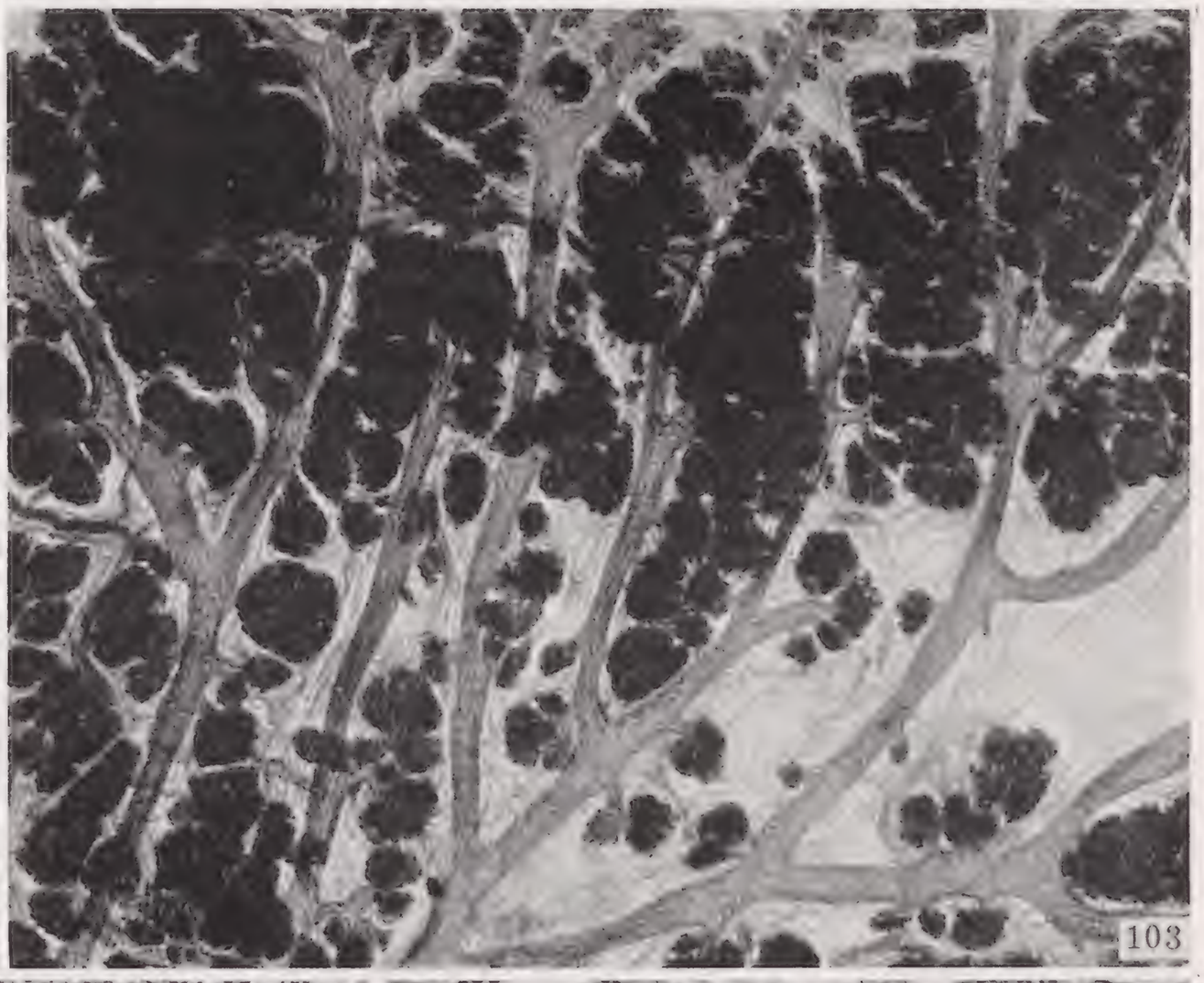

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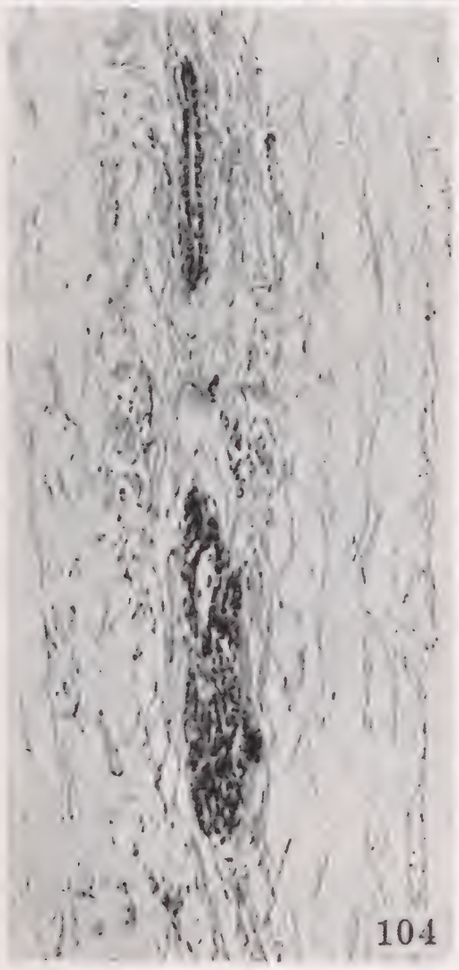

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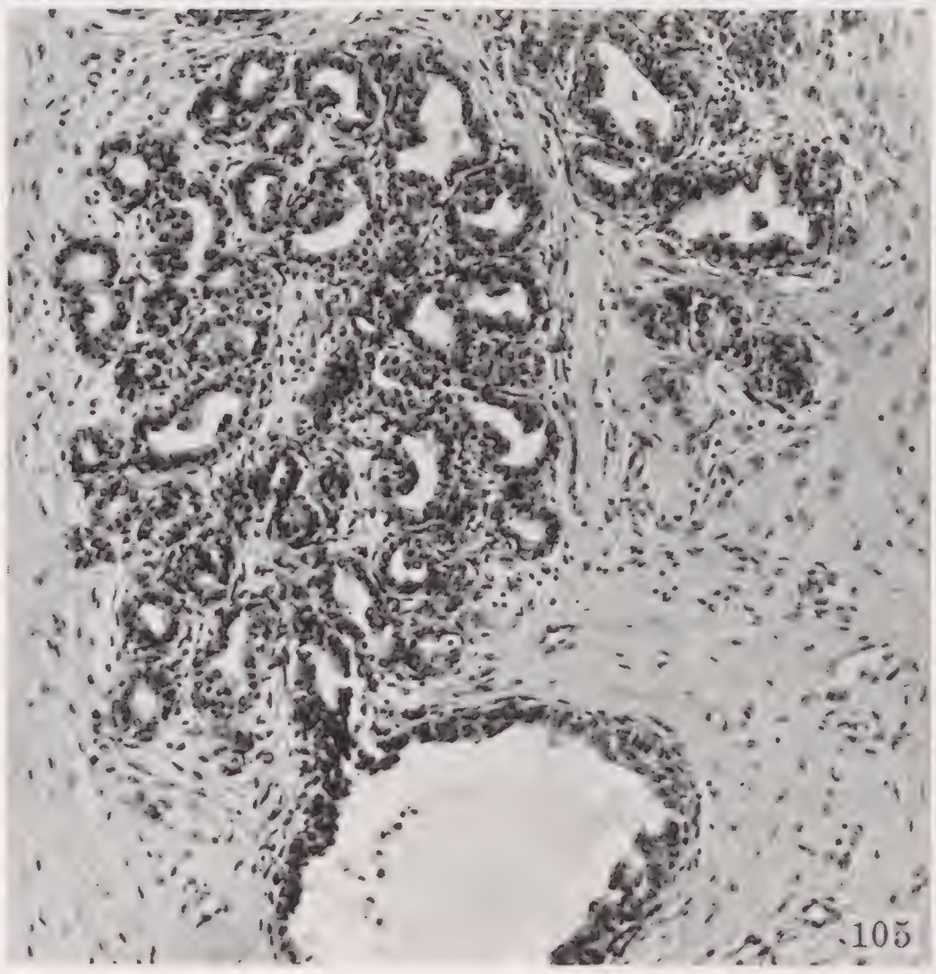

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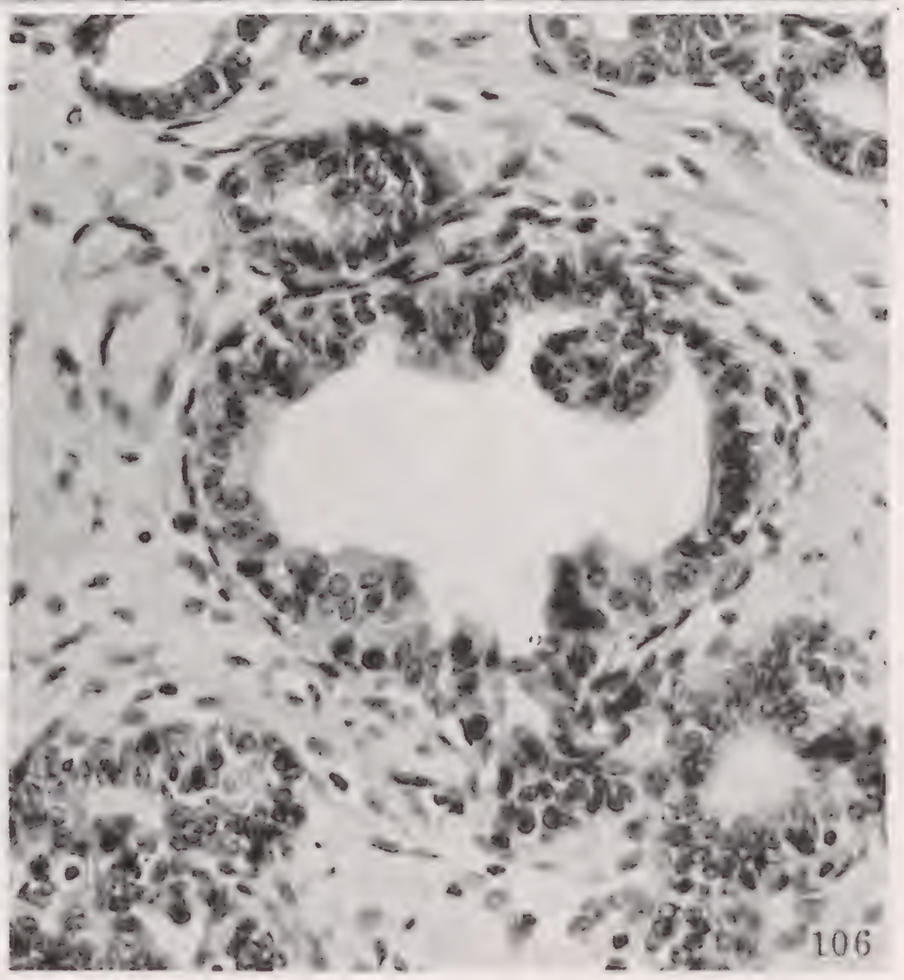

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## Plate 18

Figs. 107–110. Monkey no. 703, castrated female. Biopsy specimens of mammary gland before and after treatment with desoxycorticosterone acetate, 10 mg. daily for 24 days.

Figs. 107, 108. Gross mounts; alum cochineal; ×12.

Figs. 109, 110. Histologic sections; haematoxylin and eosin; ×100.

Figs. 107, 109. Before treatment. Gland is atrophic, alveoli collapsed.

Figs. 108, 110. After treatment, showing development of lobules, dilatation of alveoli, and prominent periductal connective tissue.

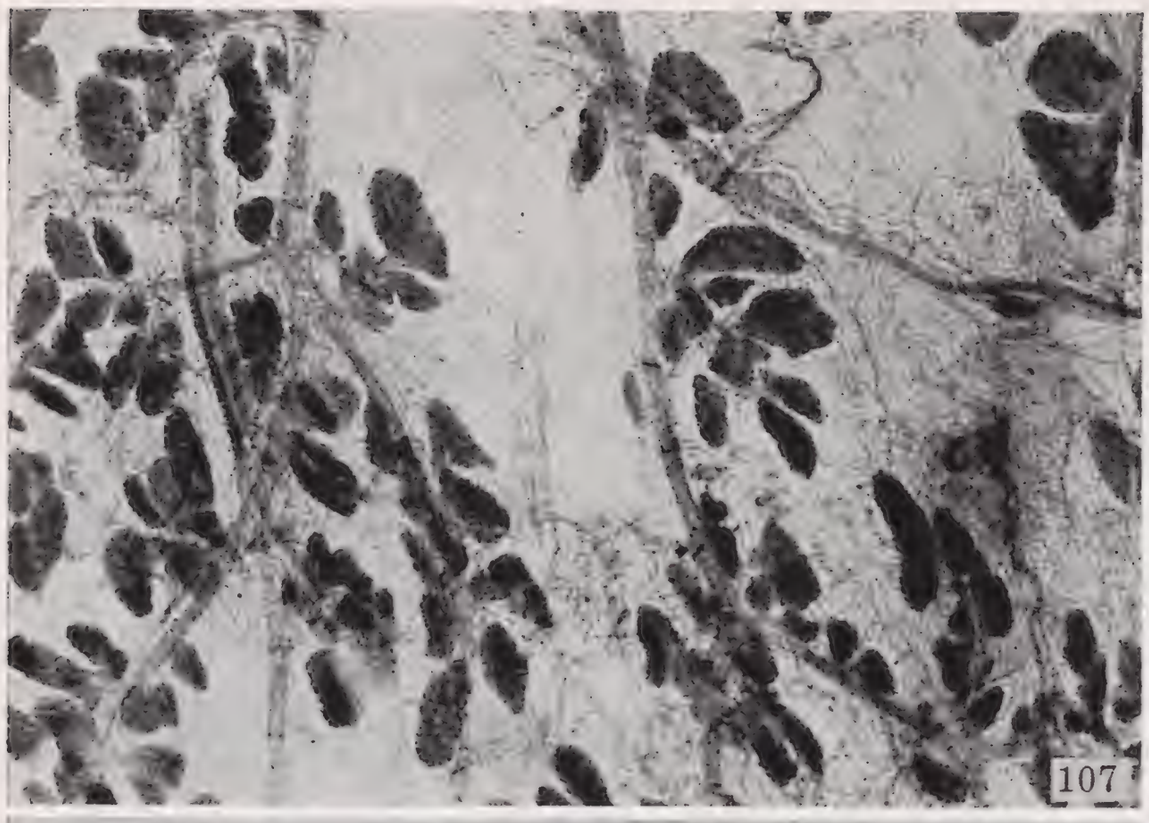
Figs. 111–114. Monkey no. Li. Mammary glands after treatment for 75 days by topical application of alcoholic solution of estrone daily to left nipple area and plain alcohol daily to right nipple area.

Fig. 111. Right mammary gland. Gross mount; alum cochineal; ×3.

Fig. 112. Left mammary gland. Gross mount; alum cochineal; ×3.

Fig. 113. Right mammary gland. Histologic section; haematoxylin and eosin; ×100.

Fig. 114. Left mammary gland. Histologic section; haematoxylin and eosin; ×100.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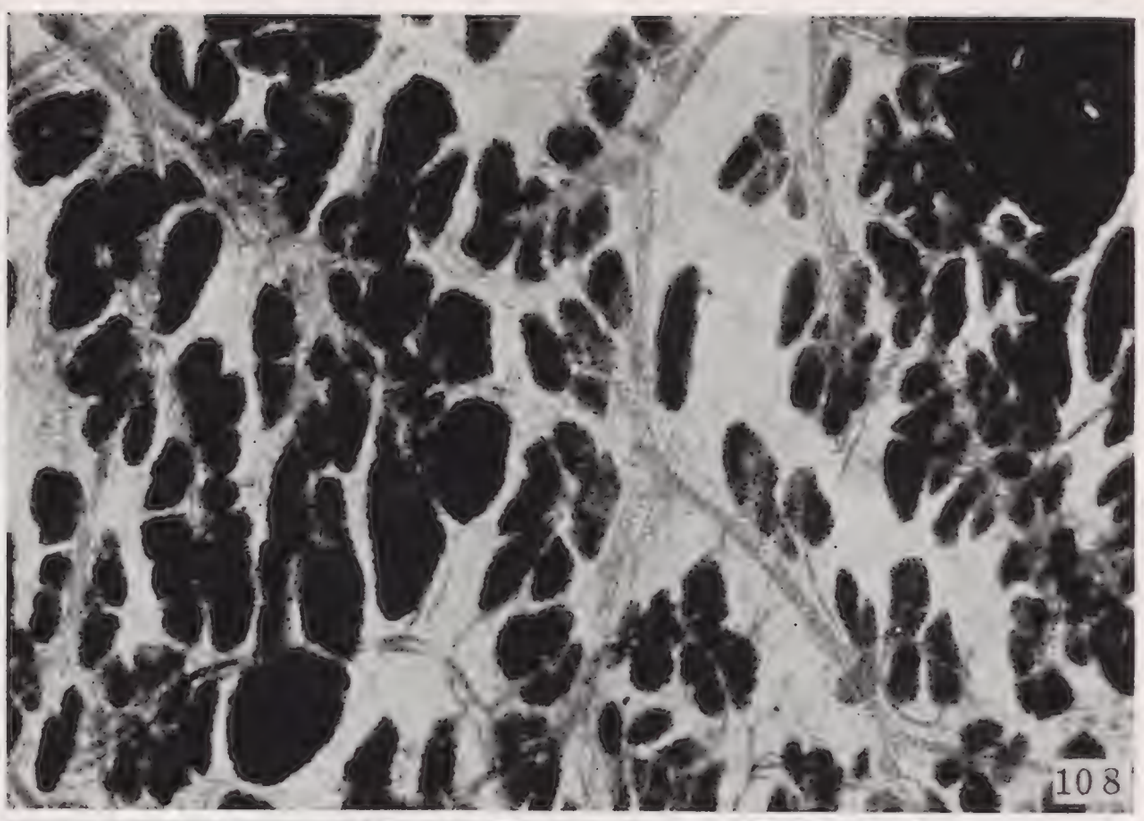
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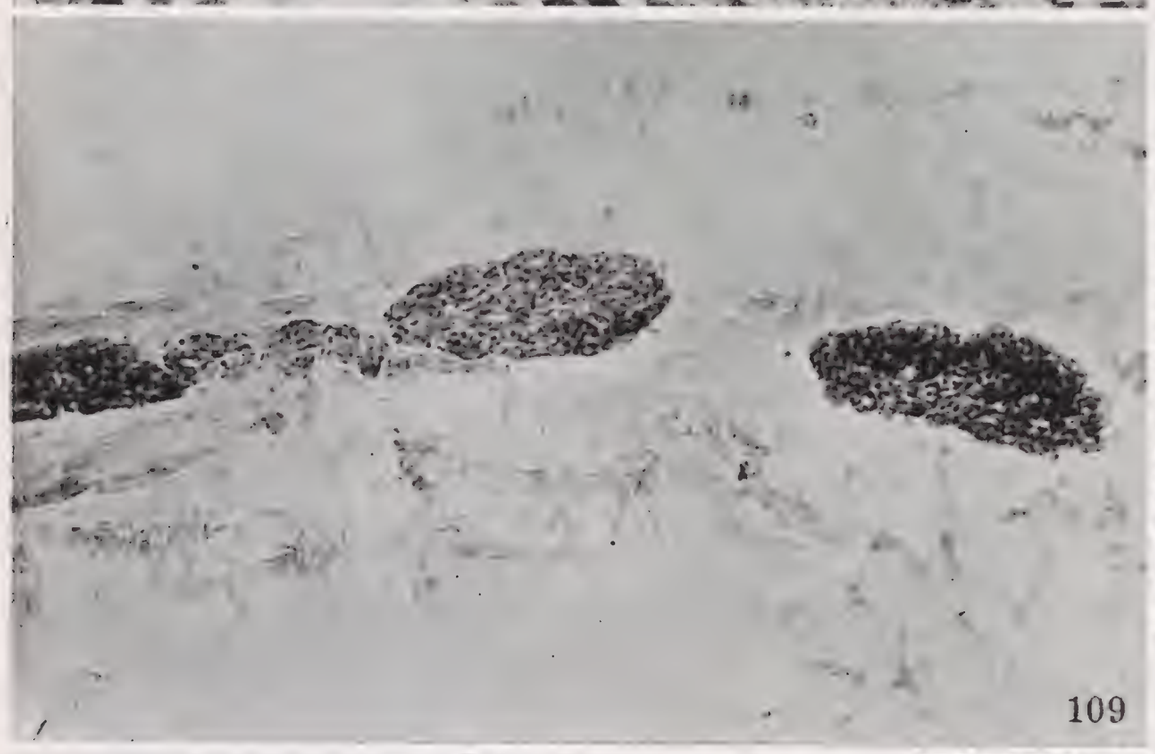
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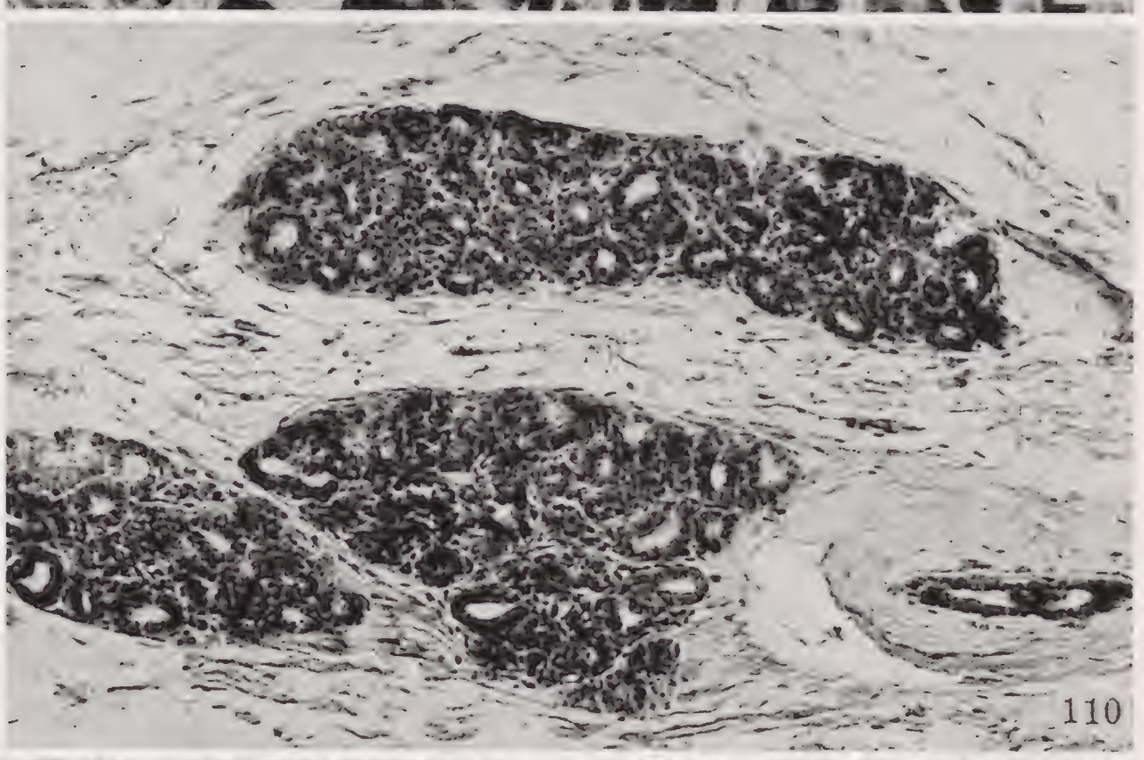
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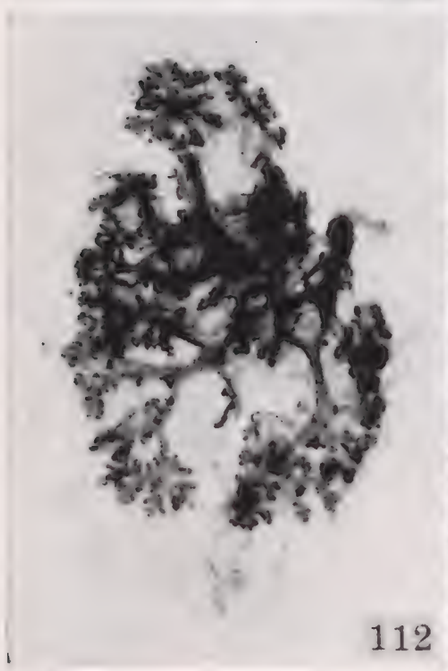
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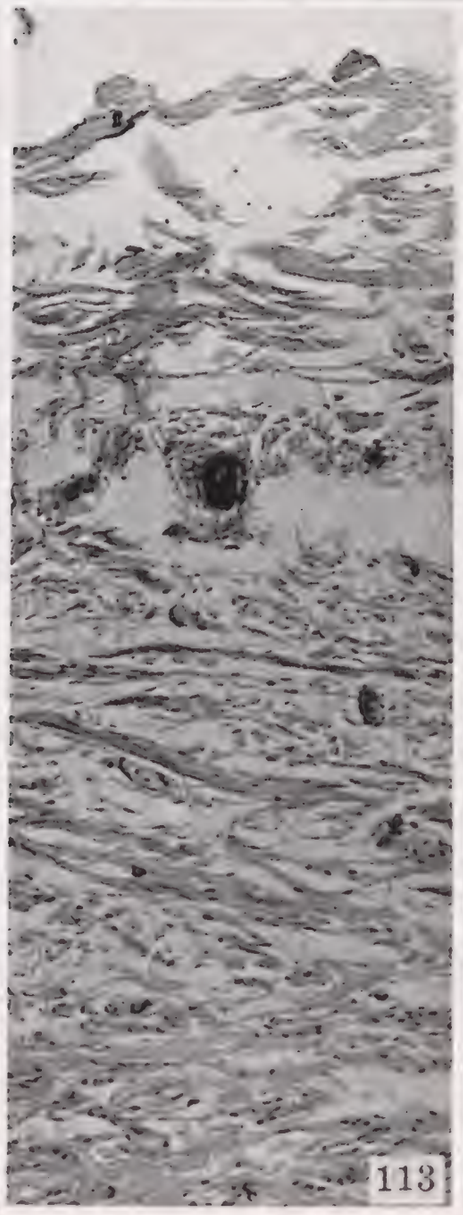
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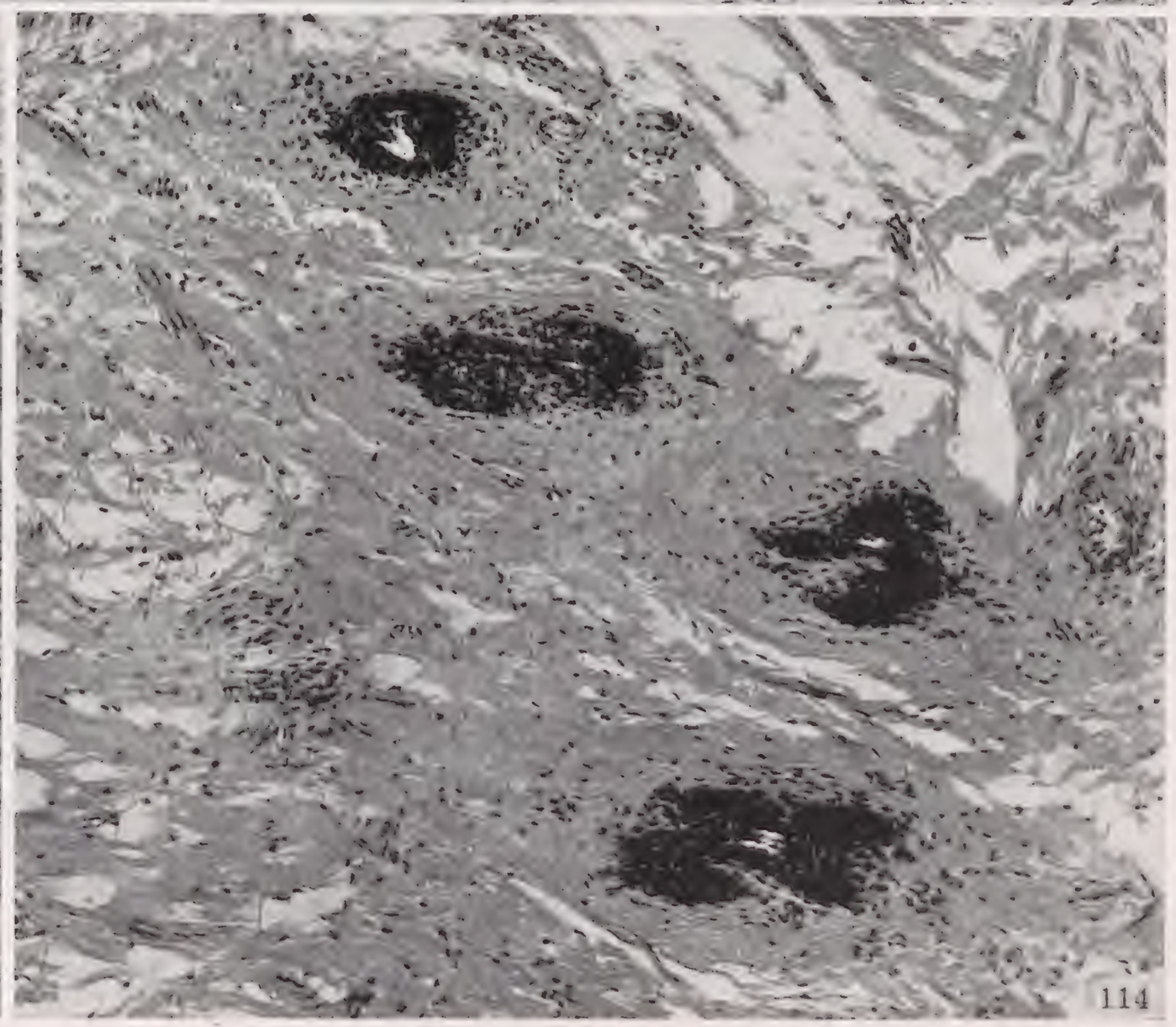
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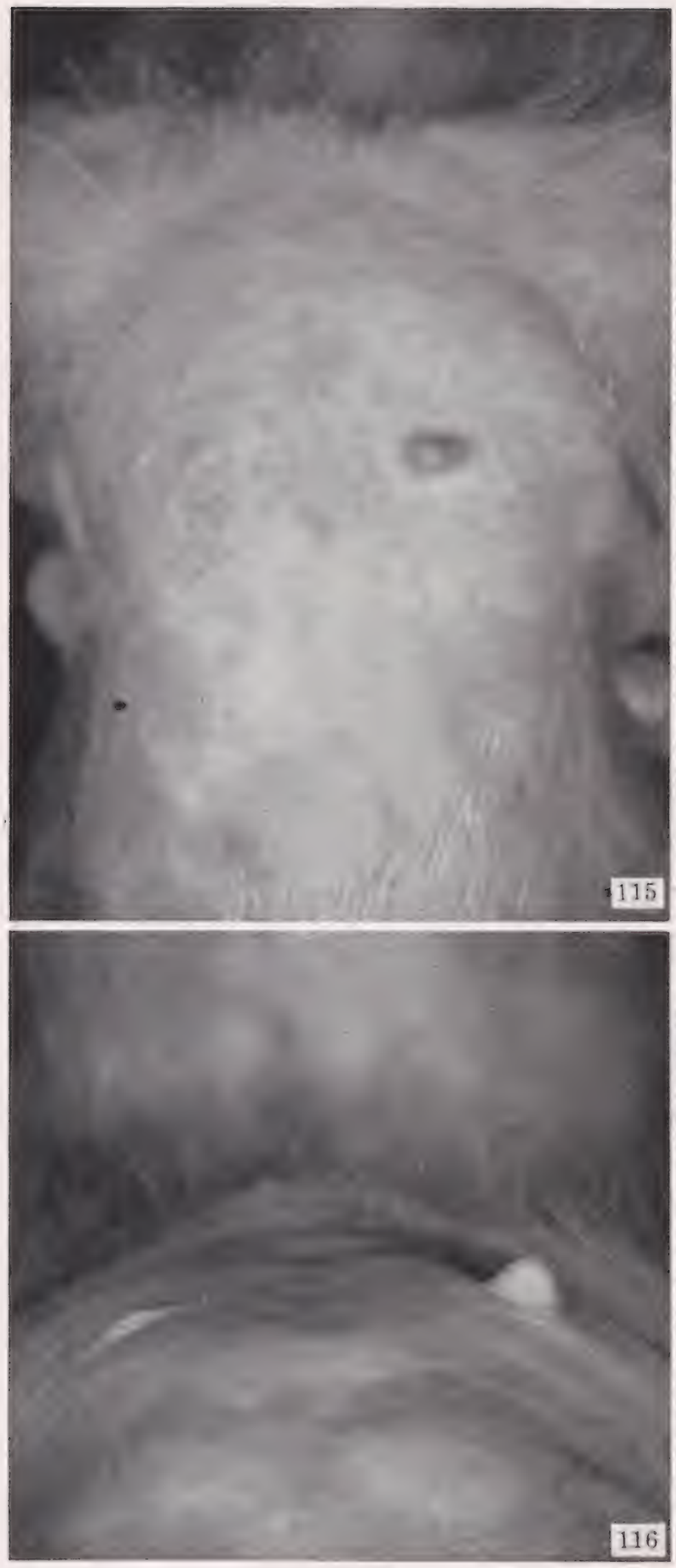
## Plate 19

Figs. 115, 116. Monkey no. L2. Nipples after 75 days' treatment similar to L1 (pl. 18). Note increased size and hyperemia of left nipple over right.

Figs. 117, 118. Monkey no. L2. Nipples after 75 days' topical treatment: left nipple with estrone, right nipple with alcohol. Sections; haematoxylin and eosin; ×100.

Fig. 117. Left nipple, showing increased thickness of epithelium.

Fig. 118. Right nipple.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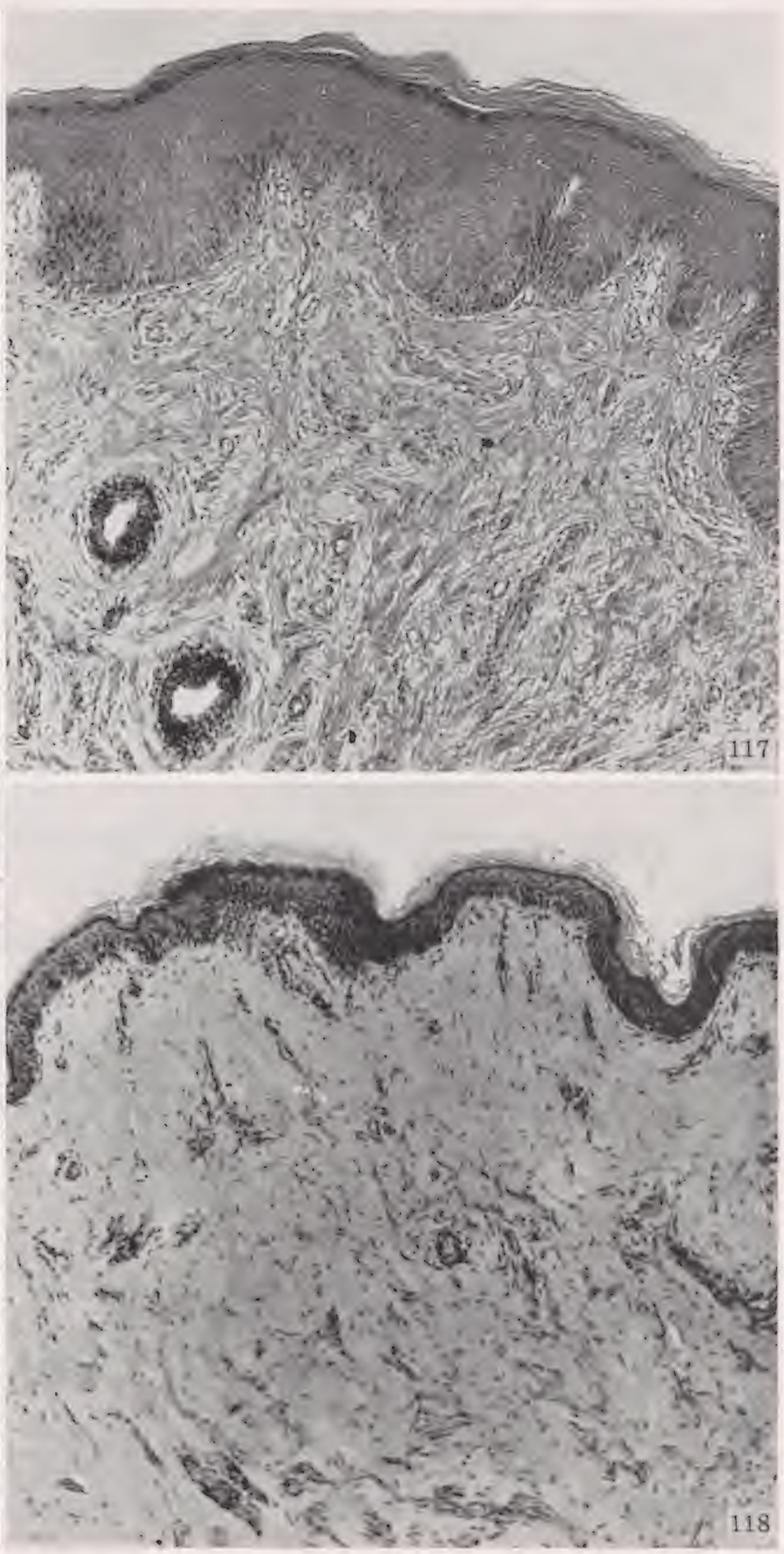
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CONTRIBUTIONS TO EMBRYOLOGY, NO. 209

# MIGRATION OF THE GERM CELLS OF HUMAN EMBRYOS FROM THE YOLK SAC TO THE PRIMITIVE GONADAL FOLDS

EMIL WITSCHI

*Department of Zoology, State University of Iowa*

With nine plates

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# MIGRATION OF THE GERM CELLS OF HUMAN EMBRYOS FROM THE YOLK SAC TO THE PRIMITIVE GONADAL FOLDS [1]

## INTRODUCTION

Extragonadal, primordial germ cells in human embryos were first seen and described by Fuss (1911, 1912) and Felix (1912). But their reports, especially that of Felix, are brief, and fail to give a comprehensive or in any sense complete description of the cytology, distribution, and migration of these cells. Nor have the succeeding years yielded supplementary studies as extensive as one might have expected in view of the keen interest taken in problems of human reproduction during this period. The most substantial contributions are those by Politzer (1928*a*, 1928*b*, 1930, 1933), which contain descriptions of the germ cells in seventeen human embryos from collections at Vienna and at Brünn.

While examining the collection of human embryos at the Carnegie Embryological Laboratory, the writer was truly surprised by the relative ease with which the primordial germ cells can be seen in the serial sections. None of these embryos had been prepared expressly for an investigation of the genital system. A large number, however, are in a general condition of fair or even excellent preservation. Practically all are perfectly sectioned, though often the staining is not very favorable for the present purpose. One can partly compensate for this factor by an adequate selection of filters and photographic materials. It is obvious that with specially adapted technical methods, considerably more cytological detail could be brought out. The thickness of the sections of the selected series ranges from 6 to 20 microns. Only the thinnest sections offer good views of the true shape of the germ cells. At 10 microns the outlines are indistinct, and at 20 microns all germ cells appear globular or ovoid.

The germ cells were counted in only a few embryos. Though the collecting of extensive statistical data is highly desirable, evidently only counts obtained from adequately prepared embryos are of permanent value. Without assurance of completeness, any variation may express differences of technique as often as actual differences in cell numbers.

Graphic reconstructions of some characteristic cases were made in order to provide an easily comprehensible picture of the progress of the germ-cell migrations. Models of the Carnegie Collection and illustrations in the paper by Shikinami (1926) were used to some extent in preparing figures 24 and 30. For expert assistance in the photographic work, I am indebted to Mr. Chester Reather.

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## DESCRIPTION OF EMBRYOS

Some data, sufficient to characterize the approximate stages of the individual embryos, are contained in table 1. No attempt was made to determine the stage at which the germ cells separate from the soma, because the available material prior to the 12-somite stage seemed not particularly favorable for such a study. As long as they are located in the yolk-sac endoderm, the germ cells differ little from associated somatic elements, and it is difficult to identify them with certainty. Evidently these earliest stages should be studied with the help of specially fixed and stained materials.

### *Embryo No. 7851, 13 Somites, 3.5 mm. Greatest Length (by Counting Sections)*

In this embryo the yolk sac is torn and only its dorsal rim, which passes continuously into the roof of the gut, is present in the sections. Consequently the full extent of germ-cell distribution remains unknown. But it is significant that the gut proper contains no gonia and that all the clearly identified ones are located in the endodermal yolk-sac epithelium.

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TABLE 1

LIST OF 23 EMBRYOS AND LOCATION OF GERM CELLS

| Carnegie no. | Streeter group | Stage | Length (mm.) | Location of gonia | Figures |
|---|---|---|---|---|---|
| 7851..... | XI | 13 somites | 3.5 | Yolk sac | |
| 8005..... | XI | 16 somites | 3 | Yolk sac and hind-gut | 1–3 |
| 7611..... | XI | 16 somites | 2.4 | Yolk sac and hind-gut | 4, 5 |
| 2053..... | XI | 20 somites; sup. neuropore closed | 3.1 | Mostly hind-gut | |
| 6097..... | XII | 25 somites; inf. neuropore closing | 3.4 | Hind-gut | |
| 5056..... | XII | 25 somites; inf. neuropore closed | 3 | Hind-gut | |
| 7999..... | XII | About 27 somites; ear vesicle with pore | 3.3 | Hind-gut | |
| 7852..... | XII | 25 to 28 somites; ear vesicle with pore; narrow yolk stalk | 3.7 | Hind-gut | 6, 7 |
| 7669..... | XIII | 29 somites; ear vesicle closed | 5 | Gut to coelomic angle | 8 |
| 8066..... | XIII | 29 or 30 somites | 5.1 | Gut to coelomic angle | |
| 836..... | XIII | 30 somites | 4 | Gut to coelomic angle | |
| 7889..... | XIII | 32 somites; aortic arches 2 and 3 | 4.2 | Gut to mesonephric folds | 9–23 |
| 7433..... | XIII | 38 somites; aortic arches 3 and 4 | 5.2 | Gut to mesonephric folds | |
| 8119..... | XIII | 38 somites; aortic arches 3 and 4 | 5.3 | Gut to mesonephric folds | |
| 7618..... | XIII | Lens placode concave | 4.8 | Gut to mesonephric folds | |
| 1380..... | XIV | 38 somites; aortic arches 3 and 4 | 5.5 | Gut to mesonephric folds | 24–27 |
| 7333..... | XIV | Lens placode concave | 6.3 | Mesentery to mesonephric folds | |
| 7394..... | XIV | Lens cup-shaped | 7.2 | Mesentery to gonadal folds | |
| 7829..... | XIV | Lens bowl-shaped | 7 | Mesentery to gonadal folds | |
| 5437..... | XIV | Lens vesicle closing | 7 | Mesentery to gonadal folds | |
| 6513..... | XVI | Lens vesicle closed, and separated from epidermis; single metanephric bulb | 7.4 (est.) | Mainly cortex of gonadal folds | 28 |
| 6511..... | XVI | Double metanephric bulbs | 8 (est.) | Cortex and medulla of primitive gonads | 29 |
| 792..... | XVI | Double metanephric bulbs; yolk stalk disconnected from gut | 8 | Cortex and medulla of primitive gonads | 30 |

*Embryo No. 8005, 16 Somites, 3 mm. (Curved Dorsally)*

(Plate 1, figures 1–3)

As in the preceding embryo, the germ cells are located near the caudal attachment of the yolk sac. But in keeping with the more advanced differentiation of the hind-gut region, many (85) are now included in the ventral and ventrolateral walls of the gut (fig. 1), mostly above the allantoic evagination. Only 24 are in the yolk sac proper. Several of them are leaving the endoderm or are already in the splanchnic mesoderm (figs. 2, 3). It is difficult to distinguish between germ cells and certain large blood-forming cells. The nuclei are nearly identical, but the cell body of the germ cells is larger, stains more lightly, and in lateral views shows a conspicuously large and dense central body (fig. 3). There remains no doubt about the separate identity of these two types of cells, since germ cells are found only in the above-described small area, whereas blood formation is actively progressing all over the surface of the yolk sac. In the total of 109 germ cells only those clearly identifiable are included. The true number is almost certainly somewhat higher.

*Embryo No. 7611, 16 Somites, 2.4 mm. (Twisted)*

(Plate 2, figures 4, 5)

This embryo is of the same stage as no. 8005 and probably is also of equal size. Its measurements are actually smaller, but this is at least partly the result of the excessive twisting of the body. In both embryos the most cranial germ cells are about at the height of the fifteenth somite. The largest number are located at the level of the unsegmented somitic cords and of the lowest part of the closed neural tube. The most caudal ones are found in cross sections which pass through the open neural tube (compare with fig. 1). Figures 4 and 5 show the cross-sectioned hind-gut immediately below the root of the yolk sac. It is easy to recognize the accumulation of the germ cells in the ventrolateral parts of the intestinal epithelium (four on the right and one on the left side in fig. 5). On the right side (actually the left side of the embryo) is a round cell with a large nucleus but relatively

small cell body in the blood vessel adjacent to the endoderm. This is not a germ cell, but a hemocytoblast (Bloom and Bartelmez, 1940). The floor of the endodermal tube at this level consists of the folded and incompletely fused epithelia of the root of the yolk sac. A transverse fold at the bottom is cut tangentially. Its cells are large, with round nuclei and deeply staining nucleoli, but of the six that lie completely within this section, only two can safely be identified as germ cells. The remainder probably are endoderm cells. Some loose bodies in the lumen of the gut are extruded and degenerating cells of the yolk-sac epithelium.

*Embryo No. 2053, 20 Somites, 3.1 mm., Upper Neuropore Closed*

Following the 16-somite stage, the root of the yolk sac rapidly becomes constricted, with the result that a thin, tubelike yolk stalk is formed in embryos of about 30 somites (fig. 9, pl. 3). Concomitantly, the hind-gut grows into a tube of considerable length, at first through incorporation of elements of the yolk-sac endoderm into its ventral wall, but later through mitotic activity and folding of the intestinal epithelium itself. Thus some germ cells of the yolk-sac epithelium are passively transferred to the ventral and lateral walls of the hind-gut. This process was at a fairly advanced stage even in the two 16-somite embryos described above, in which the yolk-sac attachment measures about two-fifths of the body length (fig. 1). In 20-somite embryos this transfer has practically come to an end, even though the root of the yolk sac still is about one-fourth of the total length. All germ cells that have not been carried into the gut endoderm have left or are now quickly leaving the yolk-sac endoderm; otherwise they would never reach the mesonephric folds. They are scattered in large numbers in the mesenchyme, ventrally to the endodermal hind-gut and around the caudal border of the yolk-sac attachment.

*Embryo No. 6097, 25 Somites, 3.4 mm., Inferior Neuropore Closing, Ear Placode Deeply Bowl-Shaped*

*Embryo No. 5056, 25 Somites, 3 mm., Neuropores Closed, Ear Placode a Nearly Closed Vesicle*

In these two embryos the germ cells are in the hind-gut between yolk sac and allantois. Near the allantois most are included in the endodermal epithelium of the ventral and lateral walls of the gut. Toward the yolk sac they are exclusively in the surrounding mesenchyme, for the most part ventrally and ventrolaterally to the endodermal tube. In the intermediate sections the expected gradual change in the proportion of endodermal and mesenchymal distribution is found.

*Embryo No. 7999, about 27 Somites, 3.3 mm., Ear Vesicle with Pore*

This embryo is mainly of interest because some of its germ cells have moved into the crest of the endodermal gut. Otherwise their distribution is much the same as in the cases last described.

*Embryo No. 7852, 25 to 28 Somites, 3.7 mm., Ear Vesicle with Small Pore*

(Diagram opposite plate 2; plate 2, figures 6, 7)

Though the somite number remains somewhat in doubt, the condition of the ear vesicles and the small size of the opening of the yolk stalk indicate that this embryo is distinctly ahead of all preceding ones in its development. Accordingly, the number of germ cells in the dorsal crest of the endodermal tube is considerably increased. The diagram is only a sketch, not a geometrical reconstruction, and illustrates the distribution of the germ cells in the gut of this embryo. In the vitelline one-third of the hind-gut all germ cells lie in the mesenchyme. In the middle part (see fig. 6) many can be seen in the endoderm, particularly concentrated in its crest. About an equal number are scattered throughout the surrounding mesenchyme. The ventral mesenchyme, which is simply the contracted covering layer of the receded yolk sac, includes a sinuous plexus of vitelline blood vessels and numerous huge hemocytoblasts that resemble germ cells. For this reason, only those germ cells which lie immediately beneath the investing peritoneal epithelium are easily identifiable (fig. 6).

The last or allantoic part of the gut shows an even distribution of the germ cells, mostly within the endodermal epithelium.

Politzer (1928*b*) has already called attention to the fact that at this stage the endodermal germ cells often seem to be enclosed in follicles, consisting of one or several flat cells with compact small nuclei (fig. 7). The subsequent history shows that these are not the final follicle cells, for they do not accompany the germ cells on their migrations toward the mesonephric

folds. The capsular formation merely indicates that the germ cells are very closely invested in the endodermal epithelium. It is unlikely that at this stage any gonia escape into the mesenchyme.

*Embryo No. 7669, 29 Somites, 5 mm., Ear Vesicle Closed*

(Plate 2, figure 8)

*Embryo No. 8066, 29 or 30 Somites, 5.1 mm.*

*Embryo No. 836, 30 Somites, 4 mm.*

If the transfer of the germ cells from the yolk sac to the gut is considered the first period of the migration, then these three embryos are at the beginning of the second period, which ends when the gonia arrive in the primitive sex glands. A few germ cells of the first and the second, and a larger number of the third embryo of this group have left the gut and reached the coelomic angles at the dorsal root of the mesentery. Most or all of them seem to be of the mesenchymal lot and to have by-passed those enclosed in the endoderm.

The endodermal wall of the hind-gut is now rapidly progressing toward the organization of a cylindrical epithelium. A distinct basement membrane is forming, by which it becomes precisely set off from the mesenchyme. The chances of the germ cells to disengage themselves seem to be lessened. Figure 8 shows an oblique cross section through the dorsal part of the hind-gut. The delicate basement membrane runs continuously over its crest. Even the topmost germ cell is solidly enclosed. This picture suggests that in their ascent toward the crest the germ cells mostly follow the inner surface of the epithelium.

*Embryo No. 7889, 32 Somites, 4.2 mm.*

(Plates 3–7, figures 9–23)

This embryo, of the American Negro race, is the most extensively studied specimen of this series. Preserved in Bouin's fluid and sectioned at 6 microns, it offers a wealth of excellent views of germ cells at the various stations along the migratory path from the gut to the mesonephric folds. Though only lightly stained with Delafield's haematoxylin and eosin, the sections are of good photographic quality.

The embryo has three well formed visceral arches, and a fourth is just beginning to take shape. Of the five pairs of pharyngeal pouches, the first three make contact with the epidermis. The first aortic arch is interrupted; the fourth is not yet complete. The third is far stronger than the second. The left and right umbilical veins are still of equal size. The umbilical arteries originate by primitive ventromedial roots.

The postovulational age can only be estimated and probably is very close to 4 weeks. For this age and stage the sex development is remarkably advanced, and the counted number of gonia (1366) is from twice to four times that found in other comparable embryos. It is impossible to venture an opinion concerning the racial or purely individual nature of such variations.

The distribution of the germ cells is shown in table 2 and in figure 9. Nearly three-fourths have left the gut and now occupy various positions in the dorsal mesentery and around the coelomic angle (figs. 10, 11). They range surprisingly far toward the cranial tip of the mesonephric folds. Though in vertebrates from the amphibians to the mammals one usually finds only few scattered germ cells above the yolk stalk and the mesenteric arteries, in this embryo about 15 per cent of the total number are located in that region. This may indicate that even at a stage as early as that represented in figure 1 the germ cells were spread over a relatively large area of the yolk sac. In keeping with such an assumption is also the proportionately small number of gonia in the endoderm (10 per cent), since only those originally located in the yolk sac directly above the allantois become included in the epithelium of the gut. Most endodermal germ cells are now at the crest of the intestinal epithelium (figs. 9, 12, 13).

Sections through the lower hind-gut contain all stages of early emigration of the germ cells from the endoderm. In figure 14, which shows a part of the same section as figure 13 at a higher power, one can see at the right two gonia which are still deeply embedded in the epithelium. Another one, located just dorsally, presses against the basement membrane and apparently was turning to the right in order to dodge the artery (a branch of the inferior mesenteric) that runs over the crest of the epithelium. At the dorsal left a fourth cell is pressing against the basement membrane, causing the latter to jut out into the surrounding mesenchyme. A clear channel running from its inner surface toward the intestinal lumen indicates the deeper position that was formerly held by this germ cell. Finally, at the very top of the

endoderm there are two gonia—only one entirely in this section—which in preparation for their departure have dissolved the basement membrane and are sending protoplasmic processes through the openings. In the case of the right cell in figure 15, the opening has become much wider and the frontal part of the cell body, with four or five pseudopodia, is just passing through it into the mesenchymal space. Before the advancing cytoplasm is a grossly vacuolated clear space into which the germ cell apparently was to slip. As far as can be judged from the morphological aspect of this material, the frontal part seems to release proteolytic enzymes which dissolve the basement membrane and prepare a path through the surrounding mesenchyme. At the same time this side of the cell body becomes of a more liquid nature and its cell membrane is only barely discernible. Here, as in all similar instances, the blood vessels are not damaged by the lytic agents. Figure 16 shows a germ cell which has just escaped from the left wall of the endoderm. A small process is still within the closing pore of the basement membrane. A light space in the epithelium indicates the place formerly held by the gonium; the larger part of this lacuna lies in the neighboring section. The germ cell has partly turned, so that its vacuolated front is now facing dorsally. At the left, in figure 12, one notices in the mesenchyme two other gonia that have freed themselves from the endodermal epithelium.

The germ cells in the mesenchyme have many different shapes which all contribute toward the creation of a picture similar to that of histolytic amoebae. Globoid cells with well defined membranes are the exception rather than the rule in this embryo. The great majority show signs of locomotive activity. If surrounded only by loose mesenchyme they usually have filiform (fig. 17) or lobate pseudopodia (figs. 18, 19), which penetrate deeply between the somatic cells. The direction of movement is not clearly indicated in all instances. Politzer (1928*b*) assumed that the central body always marks the frontal side. But it seems unlikely that motility would be restricted to one direction. Since moving cells usually are ovoid or spindle-shaped, the relatively large central body naturally has a tendency to shift to a position in front of or behind the nucleus.

TABLE 2

DISTRIBUTION OF GERM CELLS IN EMBRYO NO. 7889 (32 SOMITES, 4.2 MM.). SLIDE 2 CONTAINS THE MOST CAUDAL, SLIDE 7 THE MOST CRANIAL GERM CELLS. (SEE FIG. 9.)

| LOCATION | NUMBER OF GERM CELLS | | | | | | TOTAL | PER CENT |
|---|---|---|---|---|---|---|---|---|
| | Slide 2 | Slide 3 | Slide 4 | Slide 5 | Slide 6 | Slide 7 | | |
| Endoderm: | | | | | | | | |
| Intestine | 40 | 99 | 7 | ... | ... | ... | 146 | 11 |
| Mesoderm: | | | | | | | | |
| Gut mesenchyme | 59 | 121 | 46 | 16 | ... | ... | 242 | 18 |
| ventral | ... | (9) | (2) | (1) | ... | ... | (12) | |
| lateral | (41) | (88) | (26) | (10) | ... | ... | (165) | |
| dorsal | (18) | (24) | (18) | (5) | ... | ... | (65) | |
| Mesentery and coelomic angle | 75 | 277 | 279 | 199 | 108 | 40 | 978 | 71 |
| Total | ... | ... | ... | ... | ... | ... | 1,366 | 100 |

In the gut, the gonia often are seen in positions between the peritoneal epithelium and the mesenchyme, but seldom within the peritoneum (figs. 12, 13). On the contrary, in the dorsal mesentery and around the coelomic angle an increasing number are noticed partly or completely embedded within the peritoneum (figs. 10–13). In view of the wide distribution of such implanted gonia, it is quite improbable that all would be carried to the site of gonad formation passively by shifting movements of the peritoneal epithelia. Instead, the comparative study of the morphology of many cells of this particular specimen and of older embryonic stages indicates that individual gonia may enter and leave the peritoneum several times in succession before they reach their final destination.

Moving among the densely packed cells of the thick

peritoneal epithelium, the gonia again make extensive use of their apparent capacity for lytic destruction of membranes, of intercellular substances, and even of entire cells that block their way. The seriation of the next four pictures (figs. 20–23) is based on the previously drawn conclusion that a cell moves in the direction indicated by the local dissolution of its own membrane, increased vacuolization of cytoplasm, and formation of pseudopodia. Thus the germ cell in the upper center of figure 20, which actually is located in the mesenchyme of the mesentery, shows the first preparatory stage of peritoneal implantation. Its membrane has a wide opening through which pseudopod-like streamers project toward the epithelium. Clearly the cell is heading for the peritoneum. Figure 21 shows another germ cell which is well implanted in the epithelium of the coelomic angle. It seems to have withdrawn slightly from the location of deepest penetration. At the coelomic end of a narrow canal there is a small pycnotic nucleus of a peritoneal cell. As this is a frequent occurrence, it must be assumed that in forcing their way the gonia kill some somatic elements, the remnants of which often are pushed into the body cavity. Figures 22 and 23 show germ cells that definitely are on the way back into the mesenchyme. In both instances the places which they occupied at the surface of the peritoneum still are wide open. In the first case (fig. 22) the pseudopodia emerge from a single base, whereas in the second, at least two major processes are visible (fig. 23). Probably the right one was being withdrawn and the cell had started to turn in the direction pointed by the left branch. A cytoplasmic inclusion near the front end suggests phagocytic functions. In figures 10 to 13 many additional stages of this process of temporary association of germ cells with the peritoneum may be seen.

The farthest-traveled germ cells occupy positions under the ventromedial edge of the mesonephros (fig. 11). It is of interest that they do not enter the mesonephric blastema cords (*Segmentstiele,* funnel cords) which at regular intervals make contact with the peritoneum (figs. 10, 13). In the literature the mesonephric character of these blastema cords has sometimes been questioned and an origin by invagination or proliferation from the peritoneum has been considered as an alternative. Embryologically, the primary cords, which join the peritoneum at points near the coelomic angle, are remainders of relatively more extensive connections, characteristic of earlier stages before the separation of nephric and lateral mesoderm. The exclusive selection of the coelomic epithelium by the migrating germ cells indicates a distinct physiological difference between it and the blastema cords and, therefore, further justifies classification of the latter with the mesonephric complex. The secondary cords (fig. 13, near left edge of photograph) are even more clearly separated from the peritoneum.

The steady increase in the number of germ cells during the entire period covered by this study is sufficient evidence that mitotic activity is not interrupted during migration (fig. 10). But in view of the small number of the germ cells it is understandable that stages of cell division are rather infrequently observed.

*Embryo No. 7433, 38 Somites, 5.2 mm.*

*Embryo No. 8119, 38 Somites, 5.3 mm.*

*Embryo No. 7618, 4.8 mm.*

The first two embryos are very similar to no. 1380, described below. The last one (no. 7618) is slightly more advanced. Its body is tightly curled up, which accounts for the low measurement. The lens placodes have started to invaginate (watch-glass stage). Most of the germ cells are in the region of the coelomic angle. Very few are left in the endoderm of the gut. One large germ cell lies at the very root of the yolk stalk. It is doubtful whether it still might have found its way to the mesonephric folds.

*Embryo No. 1380, 38 Somites, 5.5 mm.*

(Plate 8, figures 24–27)

The lens placodes are distinct thickenings but have not started invagination. The first aortic arch has disappeared; the second one is regressing and possibly already interrupted. The third and fourth are fully developed, and the fifth and sixth are still in a primitive plexiform condition. The ventral roots of the umbilical arteries are much reduced and functionally replaced by new lateral roots which run around the outside of the nephric ducts. Small rudiments of metanephric ducts are present and project toward the lower ends of the nephric blastema cords. The endo-

dermal tube of the yolk stalk near its attachment to the gut shows constrictions (fig. 24) apparently formed in preparation for complete severance at a slightly older stage.

The distribution of the germ cells (table 3 and fig. 24) presents several interesting features. The total number, 451, is probably a little below the average for this stage, and appears particularly low in comparison with embryo no. 7889 (compare table 2). Actually, the given numbers are certainly too low, because identification of the cells is often difficult in sections of 20 microns. The small number in the gut mesenchyme is made up of cells which had just left

TABLE 3

DISTRIBUTION OF GERM CELLS IN EMBRYO NO. 1380 (38 SOMITES, 5.5. MM.). SLIDE 3 CONTAINS THE MOST CAUDAL, SLIDE 6 THE MOST CRANIAL GERM CELLS. (SEE FIG. 24.)

| LOCATION | NUMBER OF GERM CELLS | | | | TOTAL | PER CENT |
|---|---|---|---|---|---|---|
| | Slide 3 | Slide 4 | Slide 5 | Slide 6 | | |
| Endoderm: | | | | | | |
| Intestine | 37 | 63 | ... | ... | 100 | 22 |
| Allantois | 8 | 2 | ... | ... | 10 | 2 |
| Yolk stalk | ... | 7 | ... | ... | 7 | 2 |
| Mesoderm: | | | | | | |
| Gut mesenchyme | 1 | 18 | ... | ... | 19 | 4 |
| Mesentery and coelomic angle | 4 | 72 | 193 | 46 | 315 | 70 |
| Total | ... | ... | ... | ... | 451 | 100 |

the endoderm and were on their way into the mesentery when this embryo was preserved. In other words, all germ cells which directly ascended from the yolk sac through the mesenchyme and never were embedded in the endoderm of the gut have already reached the mesentery or the coelomic angle. In this respect the present embryo is distinctly more advanced than no. 7889. On the other hand, the number of germ cells within the gut endoderm is still fairly high and emigration seems somewhat lagging. Of great interest is the occurrence of germ cells in the endoderm of the allantois and the yolk stalk. The two groups are so located that they face each other (fig. 24), and their position leaves no doubt that they simply missed leaving the yolk sac at the proper stage. Cytologically the group in the yolk stalk still appears nearly normal, whereas the one in the allantois shows some shrinkage and other signs of regression (figs. 25–27).

In an embryo of 26 or 27 somites and a total of 586 germ cells, Politzer (1933) counted 256 within the gut endoderm. In embryos of that stage emigration has barely started. If we could assume that the number of germ cells situated in the endoderm was fairly stable and in embryos of 26 somites was near 250, emigration would amount to about 40 per cent in the 32-somite and to 60 per cent in the 38-somite embryo. Hamlett (1935) described an embryo of 4.5 mm. in which migration of the endodermal group seems unusually advanced. According to the author, "almost all" of the approximately 330 gonia are located in the mesoderm and only "possibly a half dozen are wedged among the endoderm cells."

### *Embryo No. 7333, 6.3 mm., Lens Placode Thickened and Slightly Invaginated*

All germ cells have left the endoderm and lie scattered in the mesenchyme of the dorsal root of the mesentery and along the peritoneum of the inner surfaces of the mesonephric folds. Their regular shapes indicate a more quiescent condition; some acquire follicle cells from the peritoneal epithelium. Attempts at formation of gonadal folds along the medial surface of the mesonephric bodies are noticeable. Mesonephric blastema cells, which begin to aggregate and condense in this region, initiate the formation of medullary primordia.

### *Embryo No. 7394, 7.2 mm., Shallow Lens Cups*
### *Embryo No. 7829, 7 mm., Lens Placodes Bowl-Shaped*
### *Embryo No. 5437, 7 mm., Lens Vesicle Nearly Closed*

There are still some lagging germ cells in the dorsal root of the mesenteries, though most are now in the broad, primitive gonadal folds. They are associated mainly with the peritoneum (cortex). A few lie also within the mesonephric blastema (medullary blastema) and in the mesenchyme, which becomes compressed into a narrow layer (the primitive albuginea) between cortex and medulla. Most germ cells are now smoothly rounded and provided with follicle cells.

*Embryo No. 6513, Lens Sac Closed and Separated from Epidermis, Metanephric Duct with Single Pelvic Bulb; Estimated Length 7.4 mm. but When Measured Had Shrunk to 6.6 mm.*

(Plate 9, figure 28)

*Embryo No. 6511, as Above, but Metanephric Duct with Two Pelvic Bulbs; Estimated Length 8 mm., Actually Measured 7.4 mm.*

(Plate 9, figure 29)

*Embryo No. 792, Like No. 6511, 8 mm., Yolk Stalk Has Lost Its Connection with Gut*

(Plate 9, figure 30)

Embryos nos. 6513 and 6511 are in an excellent condition of preservation, but, measured in 80 per cent alcohol, they are distinctly smaller than any other embryos of similar developmental stage. This condition seems to be due to the particular fixation and postfixation methods used. The estimated lengths given above are arbitrary, chosen by way of comparison with other embryos of the collection. No attempt will be made here to discuss principles of measurement of preserved embryos. Evidently a correct seriation is possible only on the basis of developmental stages reached by various organs (Streeter, 1942; Corner, 1943).

In these three embryos of about 8 mm. greatest length, the germ cells have reached the end of their migration and are well implanted in the cortical and medullary layers of the sex glands. In the youngest of the three, a clear majority still reside in the peritoneum (cortex; fig. 28). In the older two they are almost evenly represented in the two systems (figs. 29, 30). As may be seen in the graphic reconstruction (fig. 30), the gonadal fold containing germ cells reaches to about the middle of the mesonephric body. Above it there forms a sterile progonad with a thin peritoneal cortex and a much better developed medulla (fig. 30). Cortex and medulla remain separated by a continuous narrow albuginea which in sections appears like a fine, light cleft. Occasionally germ cells are seen in the albuginea. Probably they are crossing from the cortex to the medulla.

## DISCUSSION

### *Migration*

One of the most interesting aspects of germ-cell migration is presented by the diversity of the individual routes which are taken. Some gonia become more or less passively transferred from the yolk sac to the ventral gut endoderm; after having moved up to the crest of the tube and pierced the basement membrane, they enter the mesentery to pursue their way around the coelomic angles toward the mesonephric ridges. An even larger number of gonia leave the endoderm while still in the yolk sac and ascend directly through the mesenchyme toward the same goal. Gonia behave also quite differently while passing through the mesentery. Some associate early with the coelomic epithelium, while others proceed more deeply in the mesenchymal stroma and pass close by the dorsal aorta before they turn toward the peritoneum of the mesonephric region. During these migrations the individual germ cells behave very much like parasitic microorganisms, or such free cells as leucocytes. They are not traveling along a given path, nor do they follow recognizable leader structures, such as blood vessels or nerves. Section-by-section counts prove that they are evenly scattered over a large area (fig. 9). They proceed with little regard for obstacles, as is strikingly shown by the piercing of the basement membrane of the gut endoderm and the forcing of their way, preceding implantation, in the peritoneal epithelium. Like fertilizing spermatozoa, these primitive gonia seem to make their own paths by releasing lytic substances.

When they approach their goal, the gonia exhibit an almost unfailing capacity for selection of the tissue in which to settle. The peritoneal epithelium near the gonadal region may be entered and abandoned repeatedly, and the mesenchyme is accepted as if it were a neutral medium. The mesonephric blastema cords at first, however, are strictly avoided. Later this behavior changes rather suddenly, and in 8-mm. embryos many germ cells pass from the cortex (peritoneum) to the medulla (mesonephric blastema) of primitive sex glands. But it seems doubtful whether this last transfer occurs through active movements of the germ cells. Instead, one gets the impression that they are carried passively by sex cords. This question is closely connected with the process of sex differentiation and will not be discussed here in detail.

The pattern of the migratory movements would be hard to understand without the assumption that in some way the germ cells are guided by chemical agents. Only in a metaphoric sense might one say that the preprimordia of the gonads "attract" the germ cells. Actually one must assume that the presumptive cortex cells release a specific substance which spreads by diffusion, and that small differences in the concentration of this substance affect the gonia, which move in the direction of higher concentration (positive chemotaxis). There is no indication that any other system along the migratory path might even temporarily assist by providing orientational signals. The gut endoderm is rather an obstacle than a center toward which free germ cells might direct their movements. It must be supposed that gonia enclosed in this epithelium were caught quite accidentally during the early stages of hind-gut formation. Their migration becomes noticeably retarded (see description of embryo no. 1380). Of the gonia ascending through the mesodermal gut, a relatively high number move along the border of mesenchyme and peritoneum; but a guiding function can scarcely be attributed to the latter, in view of the fact that other cells find a way through the depth of the bulky mesenchyme. Finally, one should not overlook the fact that the germ cells never are very far from the medial edges of the mesonephric bodies. The maximal distance scarcely exceeds 0.5 mm. at any stage. Much of their migration represents compensating movements against the stream of growth that tends to carry them away from their goal. For a long time the slowest ones maintain almost constant distances of from 0.3 to 0.5 mm. In many respects the behavior of the germ cells resembles that of white blood cells, which likewise penetrate tissue barriers and often accumulate at definite places in large numbers. Moreover, the assumption that chemotropism is the guiding principle in the migration of the germ cells gains suggestive support from the observations of Chambers and Grand (1936) on chemotactic movements of leucocytes in tissue culture.

### *Relation to Embryomata*

Following a trend of thought probably first expressed by Beard (1900), almost all recent authors writing on germ-cell migration in human embryos have considered the possible significance of extraregionally scattered gonia for the origin and development of embryomata. In the present writer's opinion, no convincing or even suggestive facts in support of such a relation have been presented. In the above-mentioned few instances of germ cells which had failed to migrate (fig. 24), one gets the impression that they were regressing and that their most probable fate was final complete resorption. The development of an embryoma from germ cells would involve initial parthenogenesis. This evidently is the origin of some gonadal cysts and teratomata. But for the vast majority of embryomata in other parts of the body, the assumption that they originate by a process of abortive twinning seems more plausible and certainly better supported by observational data (Witschi, 1934).

### *Endodermal Origin*

As was stated in the opening paragraph of this paper, both Fuss and Felix should be considered discoverers of the extraregional endodermal germ cells in man and of their migration to the gonadal primordia. Nagel, who in 1889 wrote a note on extraregional germ cells, had no embryos available smaller than 12 mm., and the position of his "extraregional" germ cells is rather progonadal than extragonadal. He should, therefore, not be credited with the discovery of the extragonadal origin of the germ cells. Felix' observations were quite incomplete. In a 13- to 14-somite embryo he counted only 7 and in one of 25 somites only 12 germ cells. In both instances they were located in the hind-gut. Either the yolk sacs and other ventral parts of the embryos were missing, or Felix simply failed to recognize the germ cells outside the endodermal hind-gut. Therefore the credit for having first described germ cells in the yolk-sac endoderm of human embryos belongs to Fuss.

Politzer (1933) subjects the work of Kohno (1925) and particularly the hypothesis of mesodermal origin of the very early germ cells to severe but obviously just criticism. Florian (1931), Debeyre (1933), and Politzer (1933) published short reports on germ cells in presomite embryos, agreeing that they are included in the endodermal yolk-sac epithelium near the allantoic evagination or, as Florian says, "in the region of the cloacal membrane and the most caudal part of the yolk-sac endoderm." The photographic illustrations (Debeyre, Politzer) indicate that cytologically the germ cells are not always clearly distinguishable

from surrounding endoderm cells. Moreover, their number seems unexpectedly low in all reports. But though obviously incomplete, the facts presented suggest that the germ cells either have a common origin with the endoderm, or branch off from a part of the early endoderm (prospective yolk sac or prospective hind-gut).

### *The So-called Germinal Epithelium*

Stieve (1927) and Neumann (1929) on the basis of entirely inadequate observations tried to revive the theory of the germinal epithelium. This theory was abandoned by its original proponent when he realized that new discoveries at the beginning of the century had rendered it untenable (Waldeyer, 1870, 1906). Politzer (1933) made a careful analysis of Stieve's hypothesis of degeneration of the endodermal germ cells and of the origin of a new set by transformation of cells of the germinal epithelium, but came to the conclusion that both are invalid. Those interested in the details of the controversy are referred to the original publications. The present observations, instead of indicating degeneration, show continued mitotic multiplication of the germ cells during migration as well as after their arrival in the primitive gonadal folds. Since there exists no stage with complete disappearance or significant numerical reduction of the germ cells, and since at all times the mitotic activity appears quite sufficient to account for the observed increases, the writer considers the assumption of secondary germ-cell formation unnecessary and unwarranted.

Simkins (1928) also claimed that the germ cells arise from the germinal epithelium. But unlike Stieve and Neumann, he has not seen "any cells of peculiar size and staining reaction" in a 4.2-mm. and a 5-mm. embryo. "Even in 7 mm. embryos . . . no cells of startling size could be located either in the mesentery or in the germinal epithelium." In fact, Simkins identifies "genitaloid cells" only in 15-mm. embryos, and true germ cells can be recognized in his figures and descriptions only in fetuses of 20 mm. and longer. The question obviously arises whether Simkins' material was different from that of the other investigators. The number of human embryos between the stages of 12 somites and of 8 mm. greatest length which have been searched for germ cells is not very large. As far as the writer's knowledge of the literature shows, there were described by Felix 3, Fuss 2, Hamlett 1, Kohno 2, Neumann 2, Politzer 11, Stieve 2. Together with the 23 described in the present paper, this makes a total of 46 embryos, in which eight investigators have identified these characteristic cells. Their identity is assured, even though the authors' evaluation of their ultimate fate differs. It seems indeed perplexing that on the other hand Simkins should have chanced to obtain exclusively embryos (as many as four) which lack these cells.

### *Keimbahn Theory*

Discussions of the early history of the germ cells often deal less with actual facts than with the concept of the "Keimbahn" (the English equivalent, "germ track," never attained equal popularity). If used in a purely descriptive sense the term may not be objectionable, though certainly it is superfluous. But usually it is flavored with a generous dose of speculation. At a time when Mendel's fundamental papers already had been printed, but before they were brought to general attention, Haeckel, Rauber, Nussbaum, Weismann, and others proposed several versions of a so-called theory of inheritance that were based on the postulate of a fundamental difference between germinal and personal (or somatic) cells. Nussbaum, who as late as 1914 upheld his claim of priority in the statement of the *Keimbahntheorie,* thought the early segregation of the germ cells may explain atavisms and the conservative character of inheritance, while the fact that they remain enclosed in the soma and exposed to its influences could at the same time account for the inheritance of acquired characters. These speculations now command little more than historical interest.

### *Cellular Specificity*

But there remains the problem of the specific character of the germ cells. The fact that germ cells in some species are traceable to individual blastomeres has often led to the misconception that they are less diversified and remain more embryonic than somatic cells, or even that they are totipotent. However, they are totipotent only in the genetical sense. Ontogenetically they are as highly specialized as neurons, muscle cells, or other strictly determined elements. No uncontested evidence is available of transformation of chordate germ cells into histological elements other

than gametes. The only alteration which environmental inductive factors may bring about in them is that of developmental sex determination, that is to say, merely determination of the ovogenetic or spermatogenetic course, respectively, of gametogenesis. Totipotent cells in the embryological sense are rarely found in vertebrates that have developed beyond the four-cell stage. But they are widely distributed throughout the entire body of ascidians, as so-called residual blastema cells. In sea squirts, germ cells apparently may arise either directly from embryonic cells or—especially after regeneration and asexual reproduction—from blastema cells. Under different circumstances the latter might have furnished only somatic elements.

A better knowledge of the ontogenetic history of the germ cells of man and the other vertebrates can, therefore, be expected to serve the students of problems of embryonic differentiation and of cellular specificity more than geneticists. Since the germ cells are relatively large and often contain characteristic plasmatic inclusions, they offer many advantages for experimental analysis. At the present state of our knowledge, there exists no compelling theoretical reason for or against the assumption of a double origin of human germ cells. The invertebrates offer clear-cut examples of species with germ cells of single origin (mosaic type) and of species in which the first set of germ cells becomes supplemented by secondary sets (regenerative type). Therefore, the case of the human species can be settled only by empirical investigation of all the critical stages at which new formation of germ cells might conceivably occur. The present study merely shows that in man the germ cells of the primitive sex glands are derived from primordial germ cells, which arise long before the rudiments of the genital folds, and which in embryos of less than 16 somites are located in the yolk-sac endoderm.

## SUMMARY

1. Twenty-three serially sectioned human embryos served as the material for a study of the origin of the germ cells and of their history up to the time of the formation of the primitive sex glands.

2. The embryos investigated range from the 13-somite to the 8-mm. stage.

3. In the youngest embryo all germ cells are in the endoderm of the yolk sac near the allantoic evagination. In conjunction with reports by Florian, Politzer, and Debeyre on presomite embryos, this observation indicates that the germ cells either have an endodermal origin or arise from primitive stem cells which also are the source of the endoderm.

4. In embryos of from 13 to 20 somites, the germ cells become transferred from the yolk sac to the hind-gut. A variable number—probably always less than half—remain temporarily in the endodermal epithelium. The larger number leave the endoderm and move into the mesenchyme.

5. The second phase of migration begins in embryos of more than 25 somites. The germ cells leave the gut, ascend through the mesentery, and advance laterally toward the mesonephric folds.

6. During this phase the germ cells form lobate and filiform pseudopodia and give signs of lytic action. It is concluded that migration is accomplished by active movements of the individual cells.

7. Germ cells reach their positions in the cortex of the primitive gonads by different routes. Such diversity suggests that the course of the germ-cell migrations becomes oriented by some specific substance emitted by the peritoneum of the gonadal region (prospective cortex).

8. At their arrival in the gonadal folds the germ cells become enclosed in individual follicle cells. This marks the end of autonomous movements. In the subsequent transfer of some of the gonia into the medulla the sex cords serve as carriers.

9. During the entire migratory period the number of germ cells increases steadily through mitotic cell divisions.

10. No facts and no convincing theoretical arguments in favor of secondary germ-cell formation during the period covered by this investigation were found. The germ cells are strictly specific and do not change into somatic elements.

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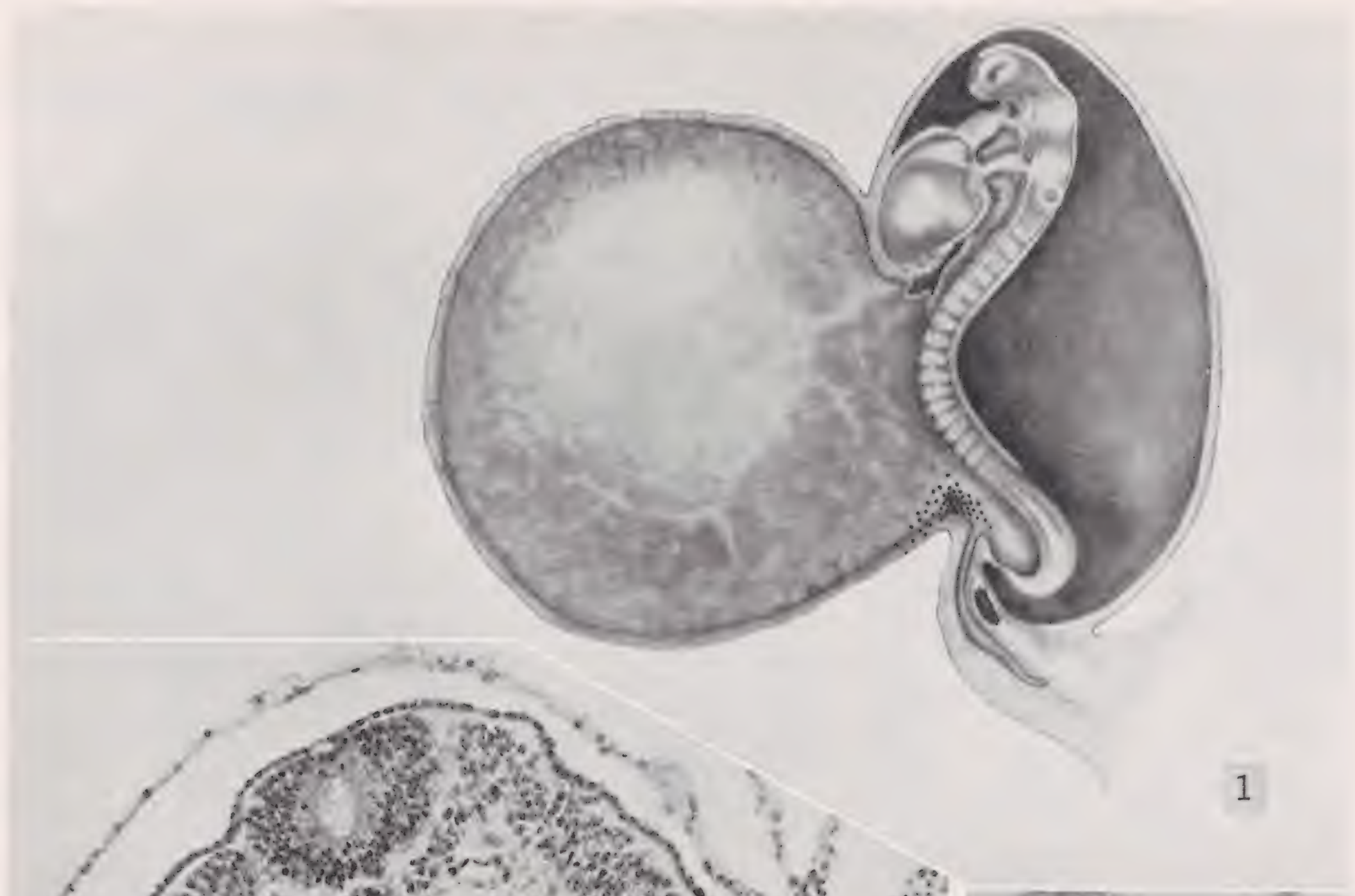
## PLATE 1

Embryo no. 8005, 16 somites, 3 mm.; sections 8 microns

FIG. 1. Reconstruction of embryo, amnion, yolk sac, and body stalk, based on photographs of the specimen and on serial sections. The germ cells (black dots) are located in the ventral wall of the hind-gut and also in the adjoining region of the yolk sac. ×22.

FIG. 2. Cross section through the embryo immediately above the lower neuropore and below the attachment of the yolk sac to the hind-gut. Germ cells are noticeable in the ventral wall of the gut and in the yolk sac. Four arrows point toward germ cells in the right wall of the yolk sac. Section 4-4-1. ×150.

FIG. 3. Detail from the same section with the four germ cells indicated above. Two are in the endoderm, two in the mesenchyme. ×600.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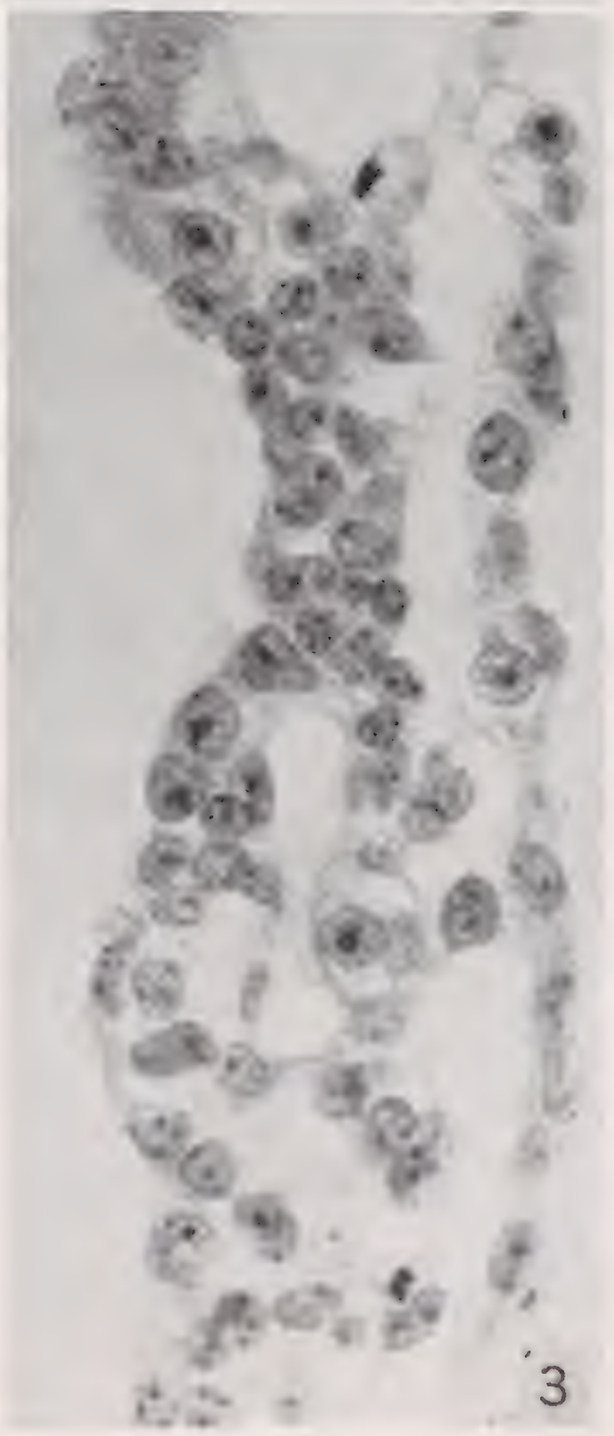
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Embryo no. 7611, 16 somites, 2.4 mm.; sections 8 microns

FIG. 4. This cross section, like the one in figure 2, cuts through the lower periphery of the yolk-sac attachment; but it passes through a higher level of the neural tube and misses the yolk sac proper. Germ cells are seen in the lateral and ventral endodermal epithelium of the hind-gut and in the ventral mesenchyme. Section 5-4-7. ×168.8.

FIG. 5. Enlarged view of the ventral wall of the gut with germ cells in endoderm and in mesenchyme. Section 5-4-7. ×600.

Embryo no. 7852, 25–28 somites, 3.7 mm.; sections 10 microns

FIG. 6. Cross section through embryo at the level of the hind-gut, showing the massive accumulation of mesenchyme in the ventral wall of the gut. Germ cells (light areas with dark central spot) are numerous in the mesenchyme, along the splanchnic peritoneum, and in the endodermal epithelium of the gut. Section 4-1-15. ×112.5.

FIG. 7. Part of a similar cross section showing germ cells in the gut endoderm surrounded by temporary follicle cells. Section 4-2-6. ×450.

Embryo no. 7669, 29 somites, 5 mm.; sections 6 microns

FIG. 8. Germ cells in the endoderm of the gut of a slightly older embryo, accumulating at the crest. A distinct basement membrane now separates the endoderm from the mesenchyme. Section 9-1-5. ×450.

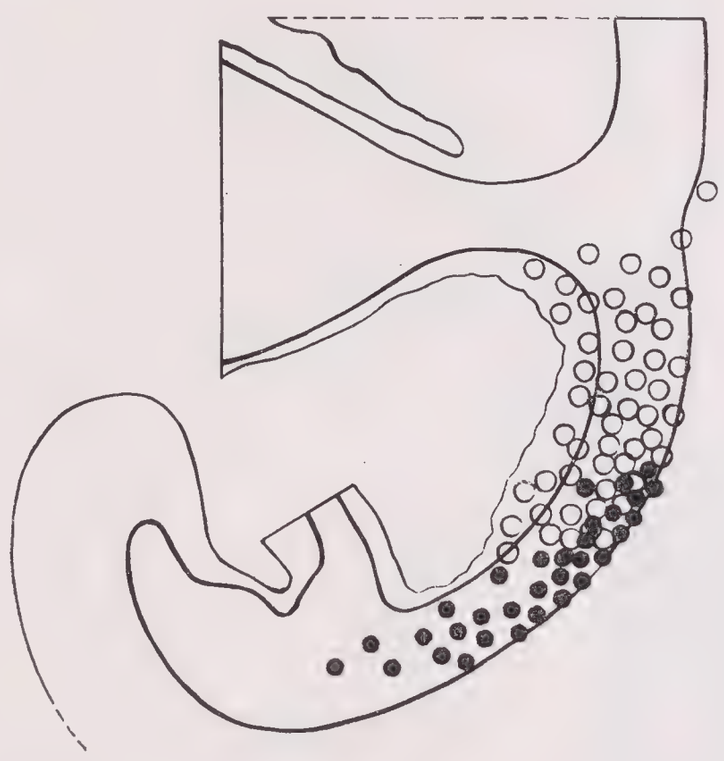

Diagram of the caudal end indicating the distribution of endodermal (dots) and mesenchymal (circles) germ cells in the gut of embryo no. 7852.

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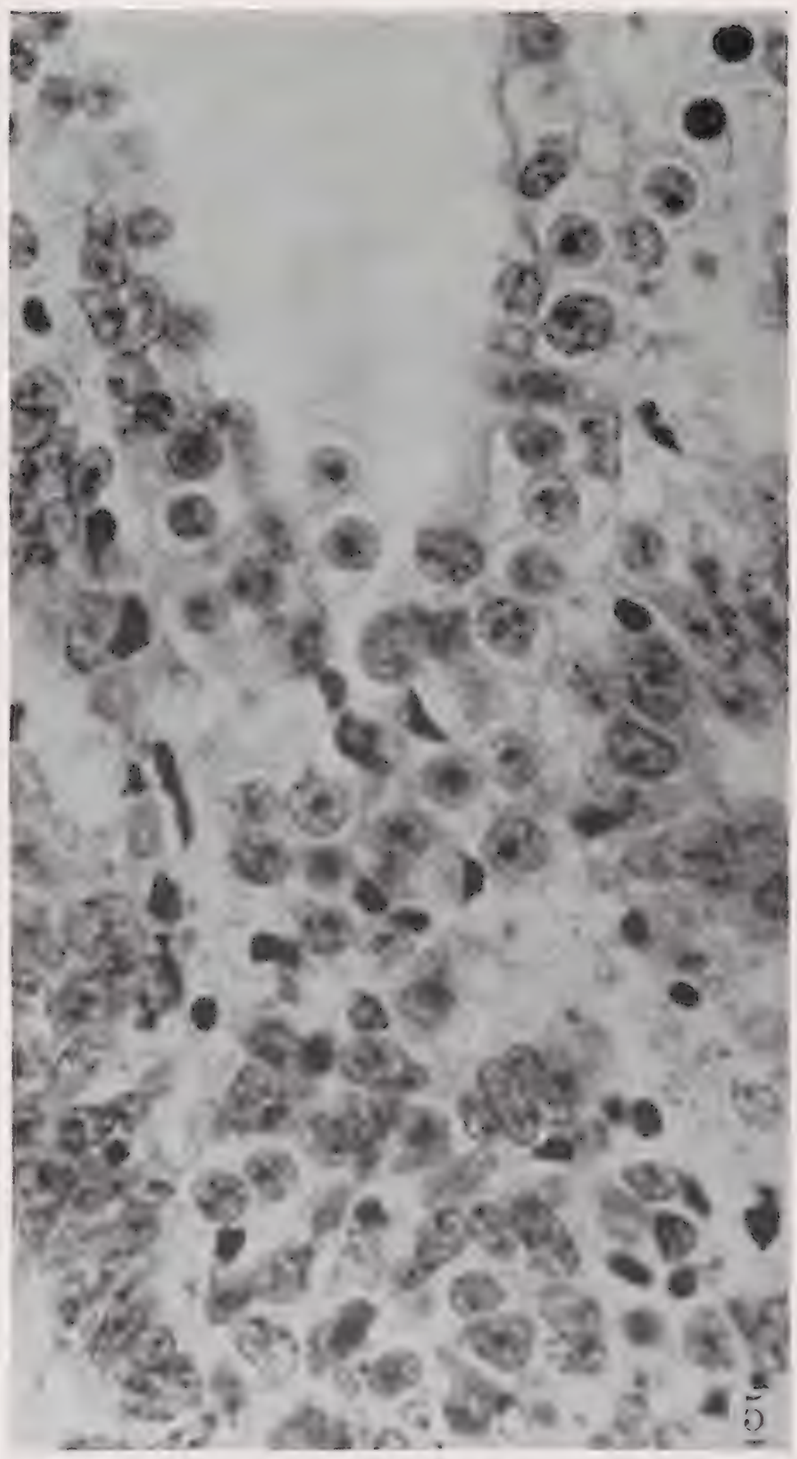

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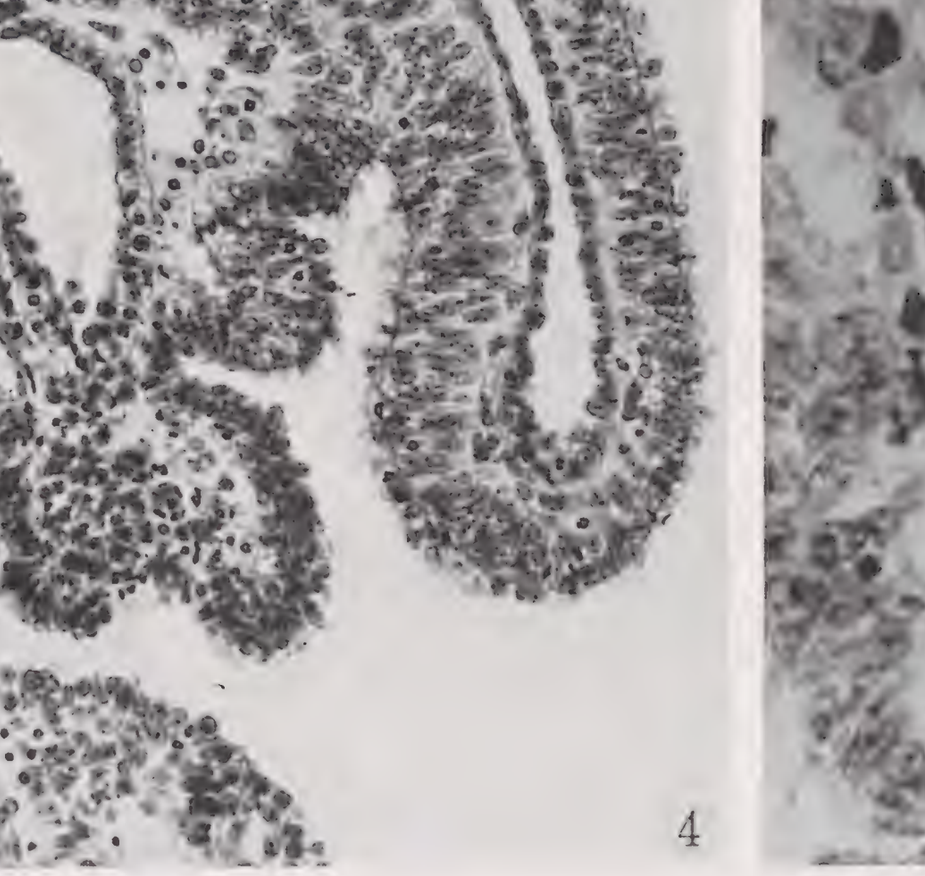

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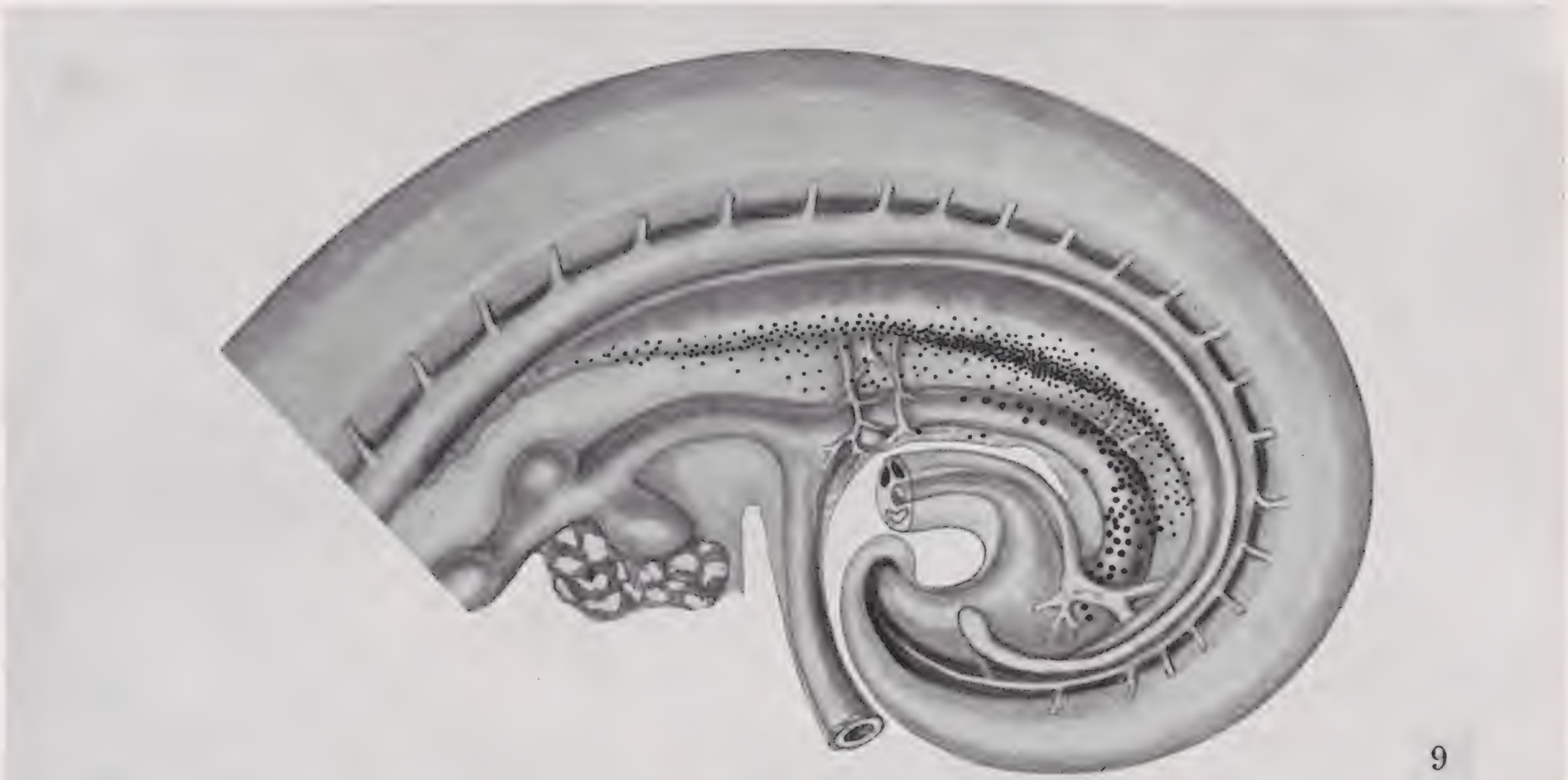
Embryo no. 7889, 32 somites, 4.2 mm.; sections 6 microns

FIG. 9. Graphic reconstruction of the left half of the lower part of the embryo; somites and body wall omitted. The descending aorta (with roots of segmental arteries) is partly hidden beneath the mesonephric body and duct. Consequently the roots of the upper and lower mesenteric and of the umbilical arteries are not visible. In reconstructing, the gut was shifted slightly in the ventral direction so as to expose the entire mesentery (the dorsal part would actually be hidden by the mesonephric fold). The germ cells are represented by black dots, the larger ones indicating an endodermal position. Few germ cells are left in the ventral mesenchyme. For the sake of clearness, the mesodermal germ cells along the left side of the endodermal tube of the gut are omitted. ×40.

FIG. 10. Cross section showing germ cells in the dorsal mesentery and around the right (partly also the left) coelomic angle. The section cuts through the hind-gut just below the upper mesenteric arteries, i.e. at a level where the endoderm (partly visible at bottom of figure) contains no germ cells. Part of a mesonephric tubule is visible at the right upper corner, and strands of mesonephric blastema project from its medial surface toward the peritoneal epithelium. There are four germ cells in the mesenchyme, five more or less embedded in the peritoneum. Section 4-2-3. ×500.



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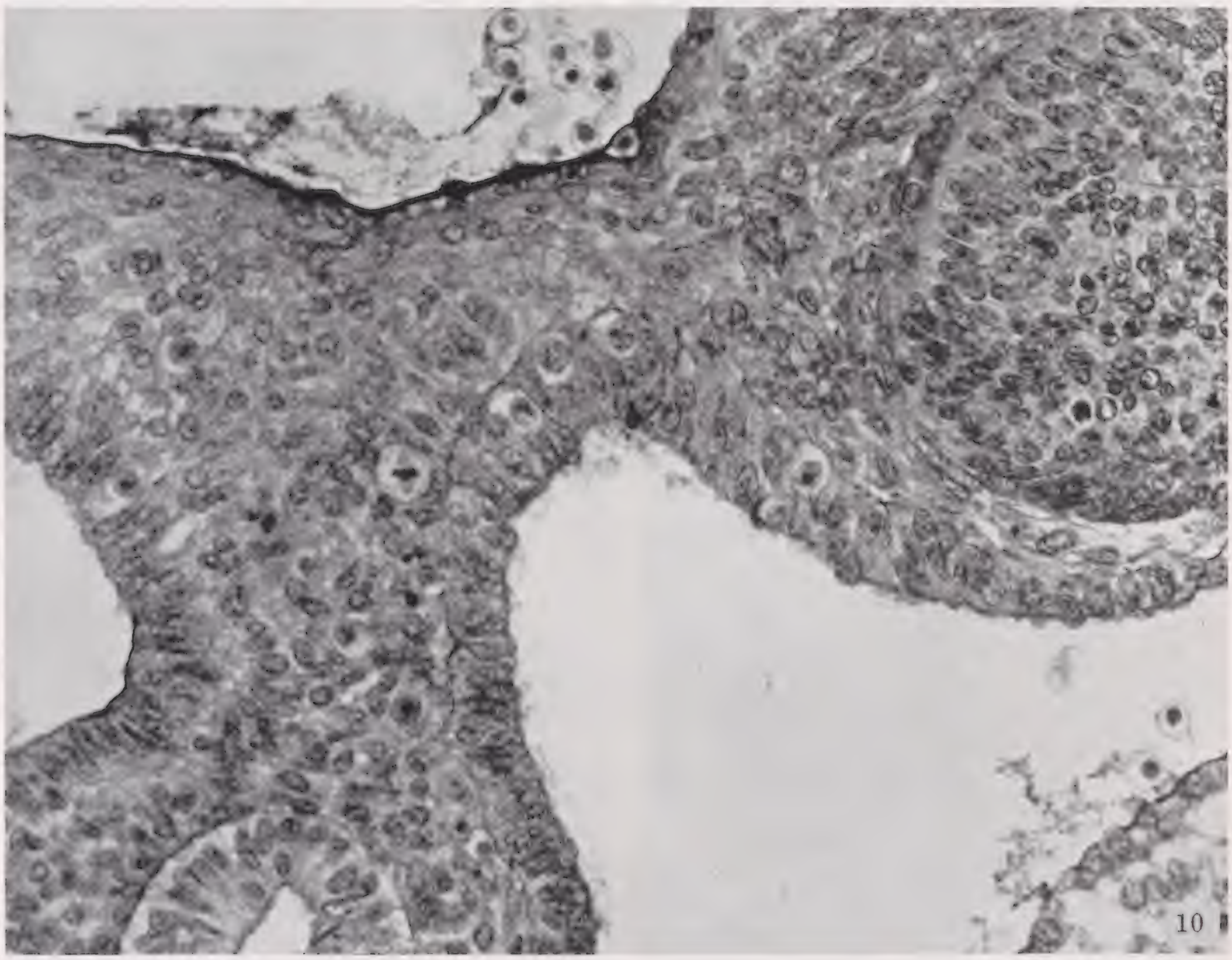

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## Plate 4

In all four figures the dorsal direction is to the left of the plate. Left and right of each section corresponds to the left and right sides of the embryo.

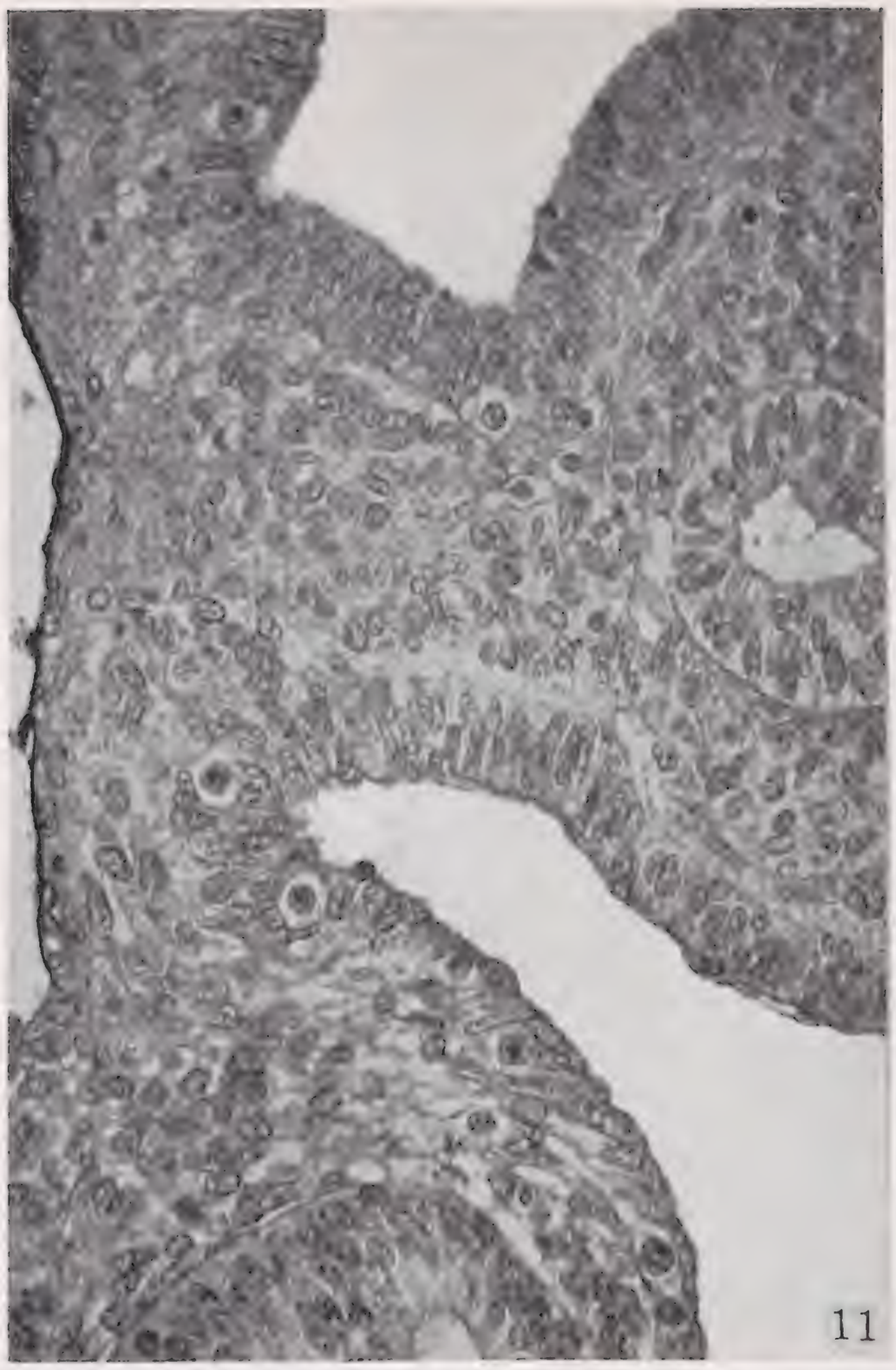
Embryo no. 7889, 32 somites, 4.2 mm.; sections 6 microns

Fig. 11. Cross section at a slightly lower level than figure 10. There are no germ cells in the endoderm of the gut. Some germ cells have advanced far around the coelomic angle and occupy the full width of the peritoneum, which later covers the gonadal fold. Section 3-4-2. ×375.

Fig. 12. Cross section through the lower part of the gut, with many germ cells in the dorsal endodermal epithelium. Other gonia are present in the mesenchyme of the gut, in the mesentery, and at the coelomic angles. Note that germ cells may fully enter the peritoneum of the mesentery. Section 3-2-6. ×375.

Fig. 13. Next section to that shown in figure 12. Above the left coelomic angle there are four germ cells in various stages of passage along the peritoneal epithelium. They avoid the mesonephric blastema cord, which here is in broad contact with the basement membrane of the peritoneum. Near the lower edge of the figure a second blastema cord similarly approaches the peritoneum. Section 3-2-7. ×375.

Fig. 14. Detail of the same section showing early stages of the emigration of germ cells from the endodermal gut (see text). ×1125.



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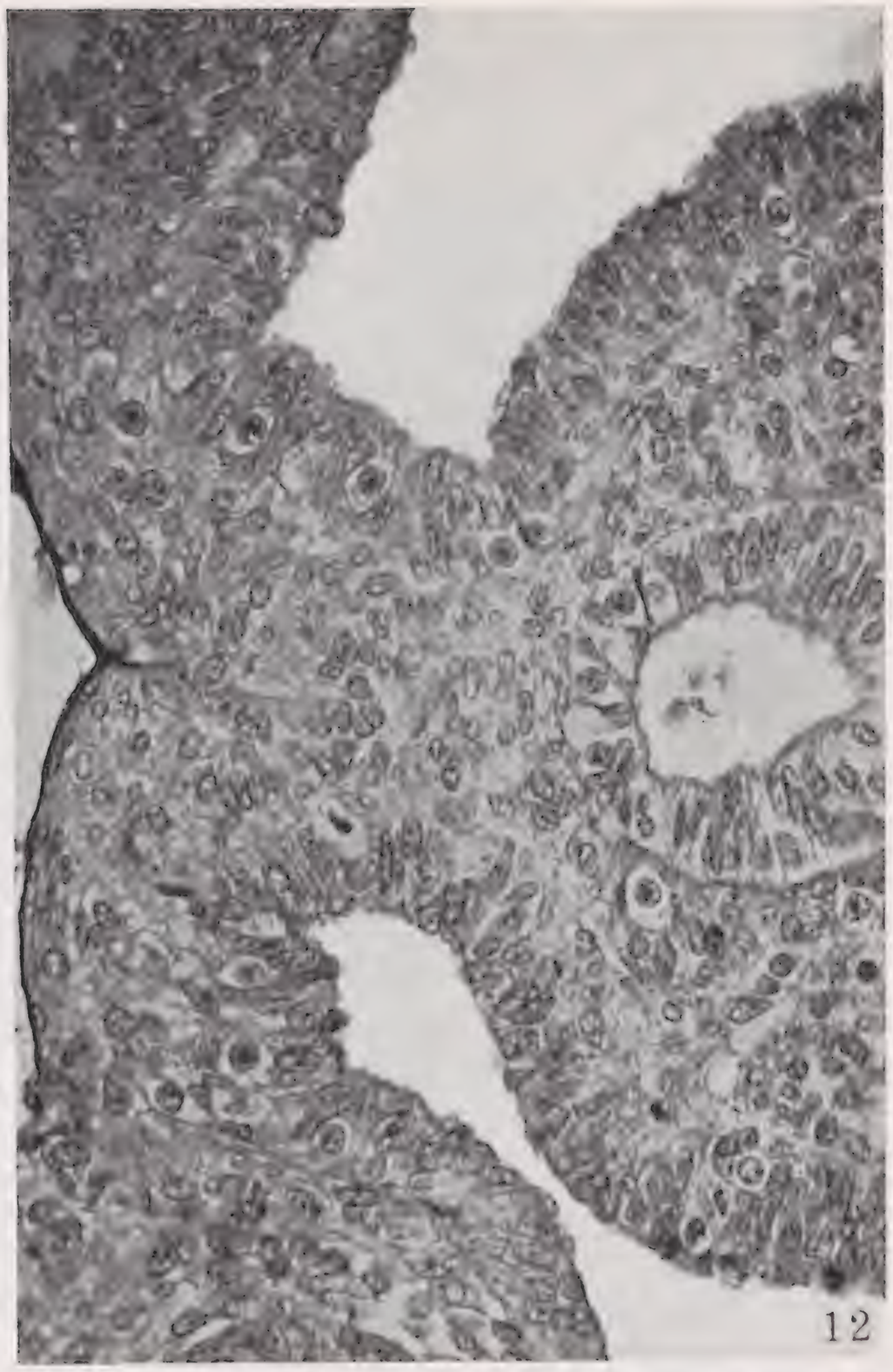

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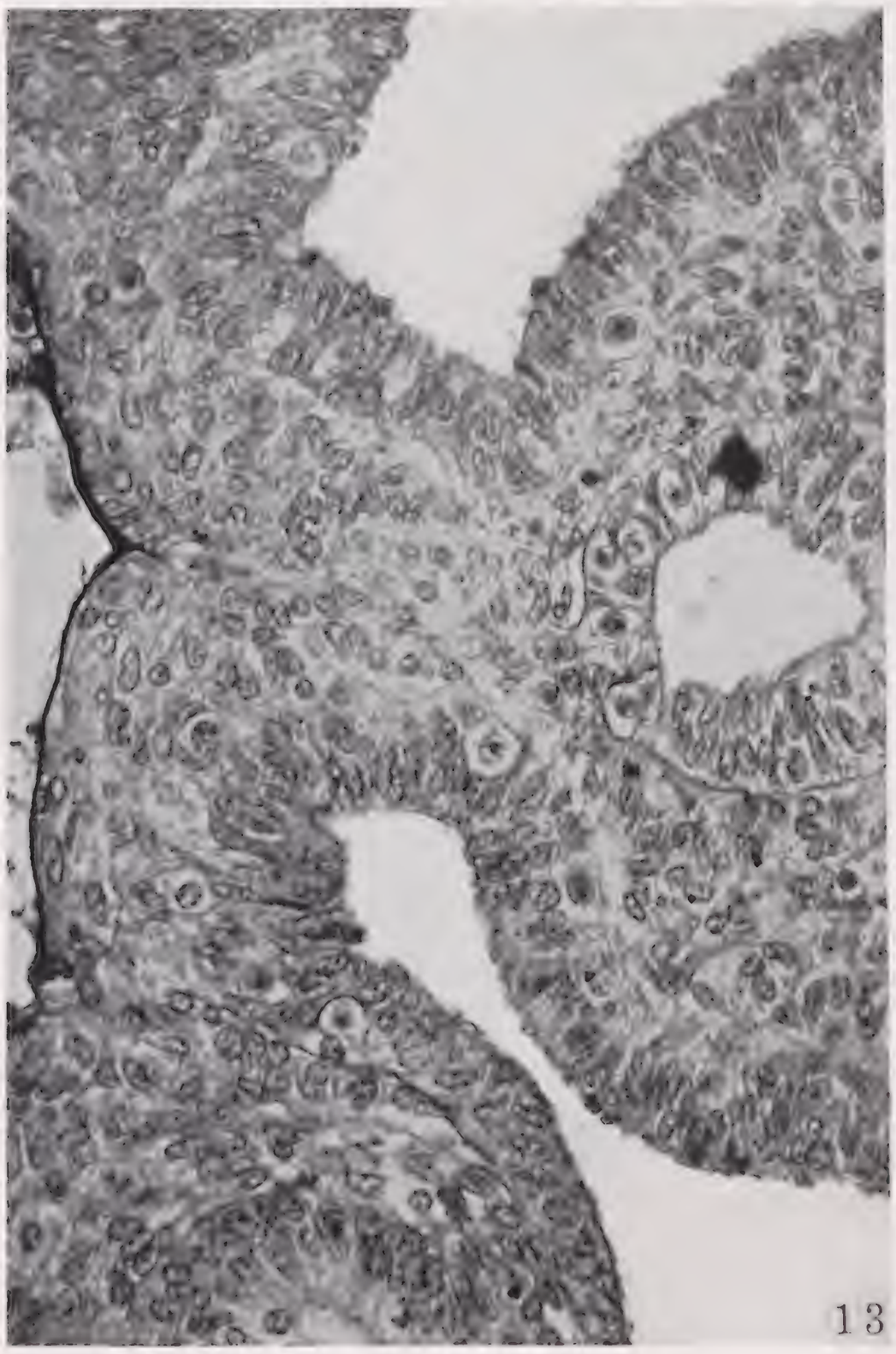

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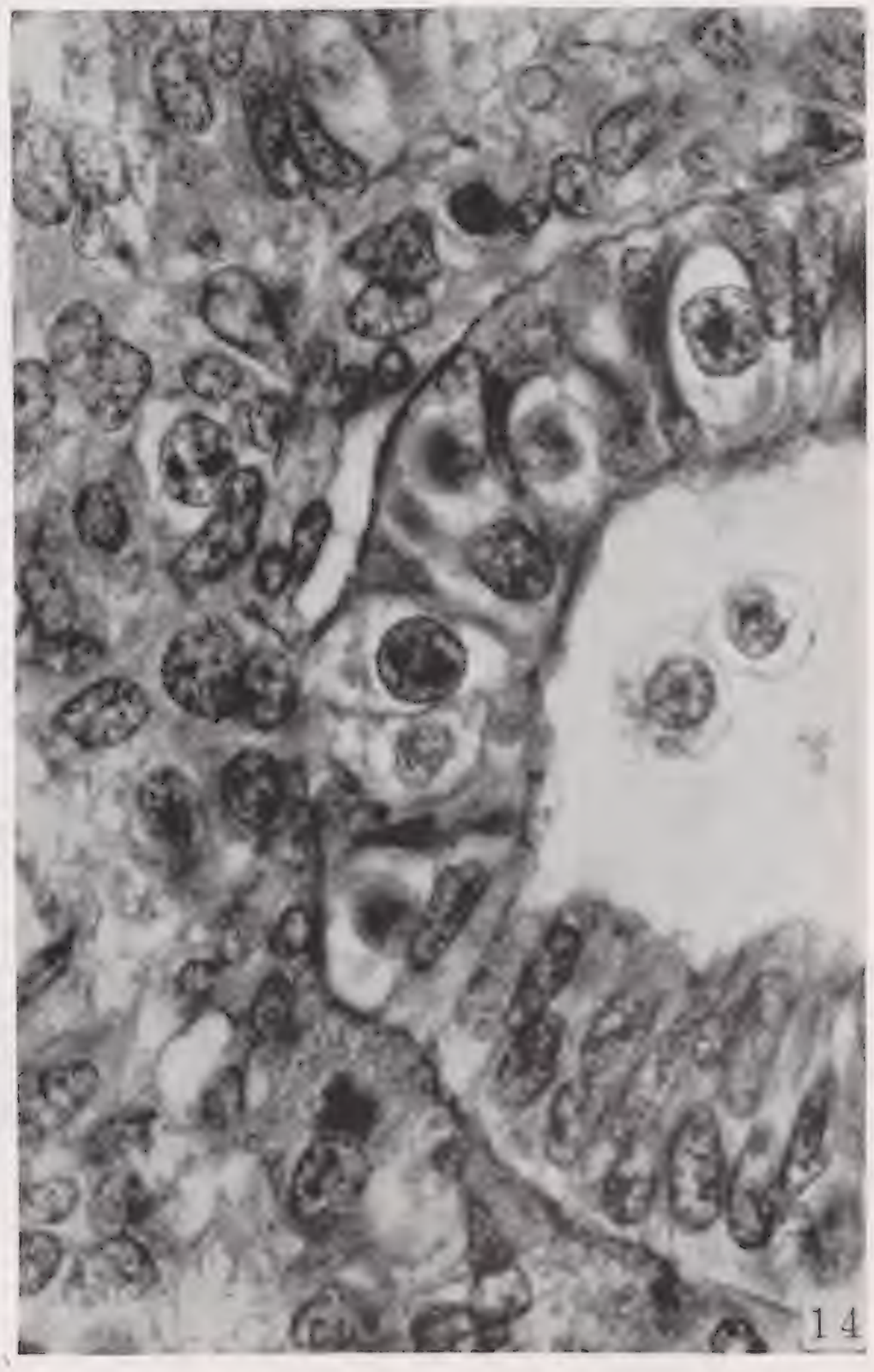

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## Plate 5

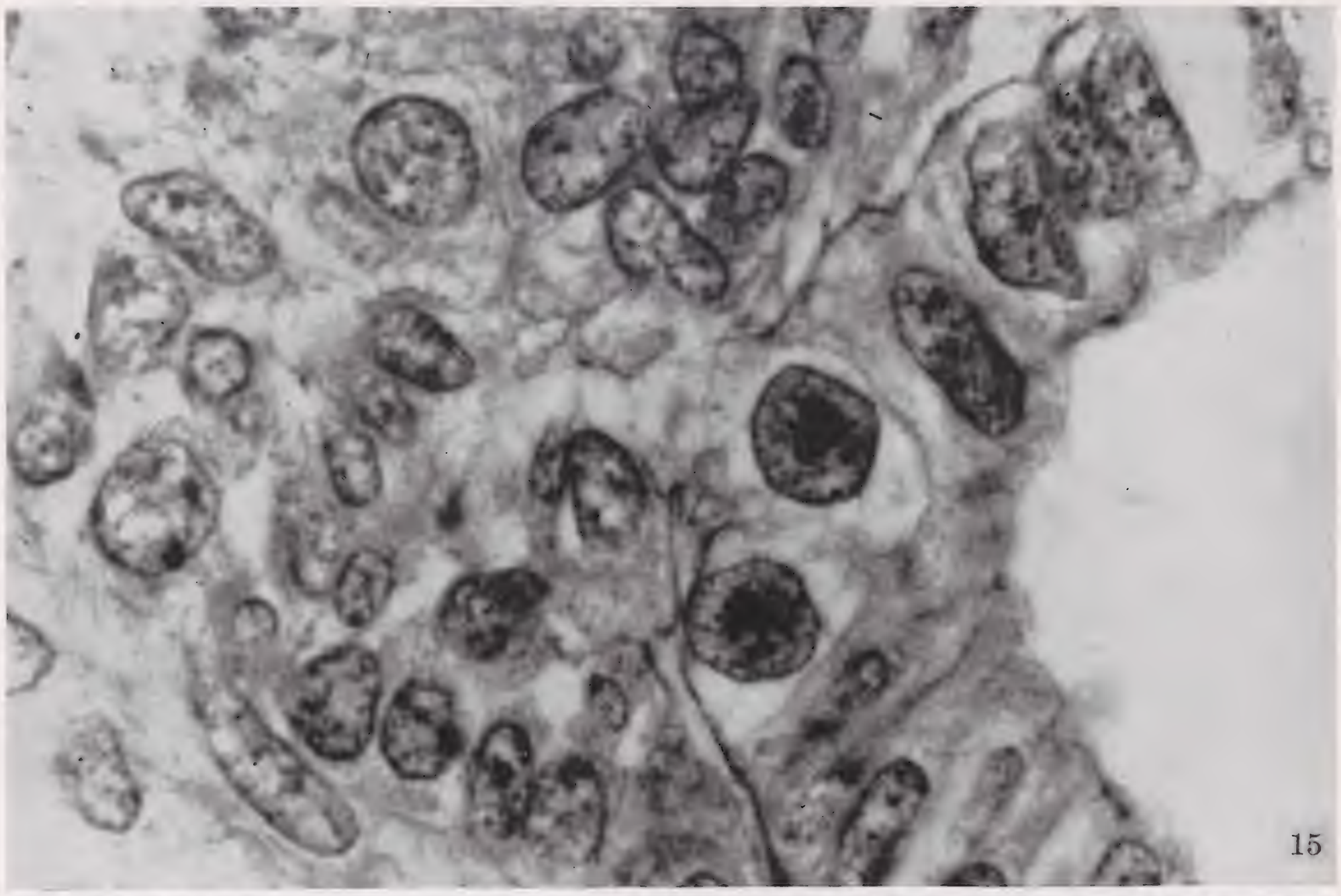
In all three figures the dorsal direction is to the left.

Embryo no. 7889, 32 somites, 4.2 mm.; sections 6 microns

Fig. 15. Section through the same region as figure 14, showing two endodermal germ cells of which the right one has dissolved a stretch of the basement membrane. Four or five pseudopodia reach out through this opening into the highly vacuolated mesenchymal tissue, dorsally above the endoderm. Section 3-2-2. ×2000.

Fig. 16. Section through the same region, with a gonium that has just passed from the left dorsal endoderm into the mesenchyme of the gut. Its former position is still indicated by a light area (mostly in next section) within the endoderm. Section 3-1-12. ×1000.

Fig. 17. Part of the gut, with peritoneum at the bottom, endoderm at the top, and mesenchyme in the center. A highly polymorphic germ cell ascends along the boundary of peritoneum and mesenchyme. Section 3-3-1. ×1500.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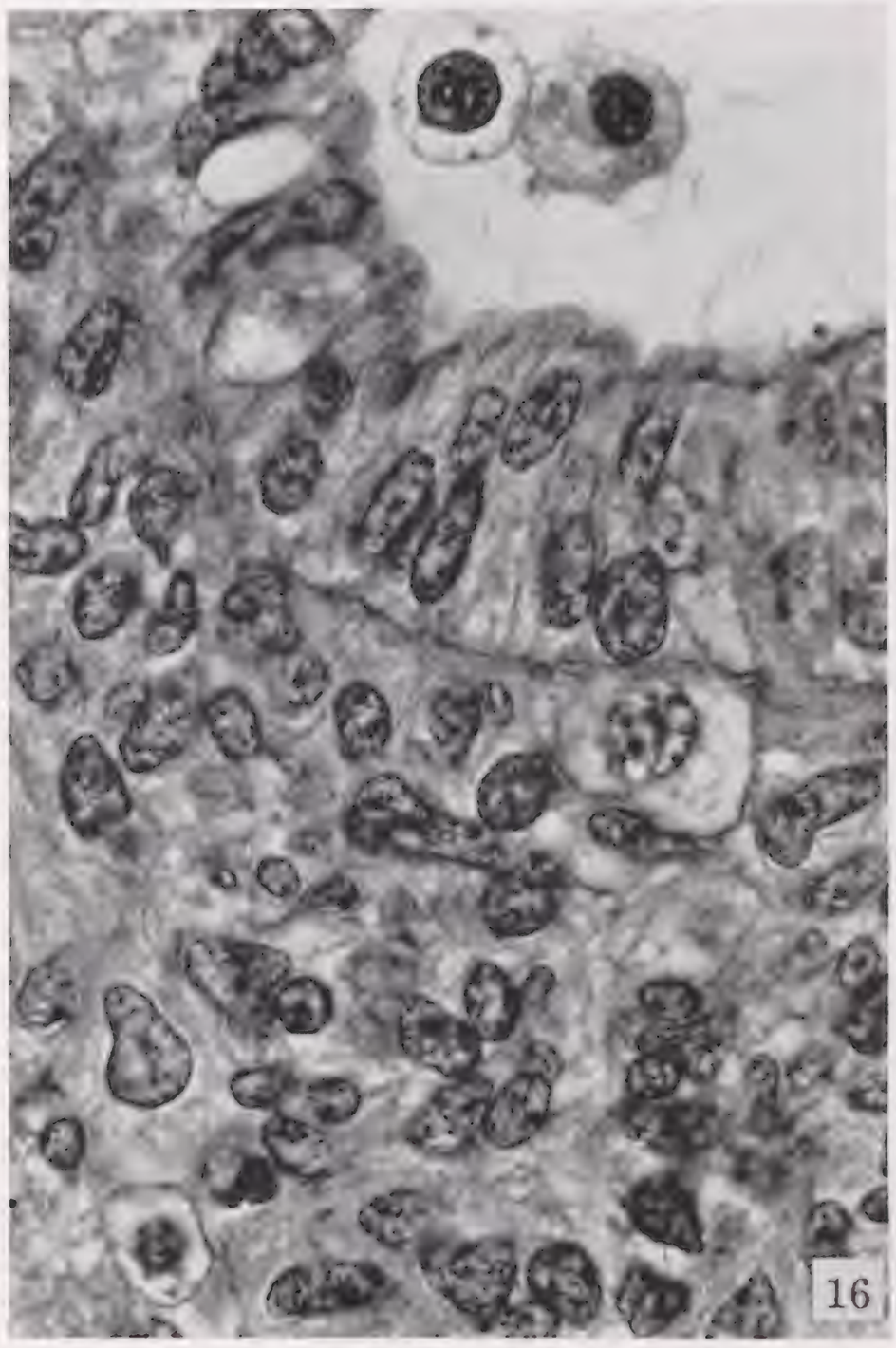
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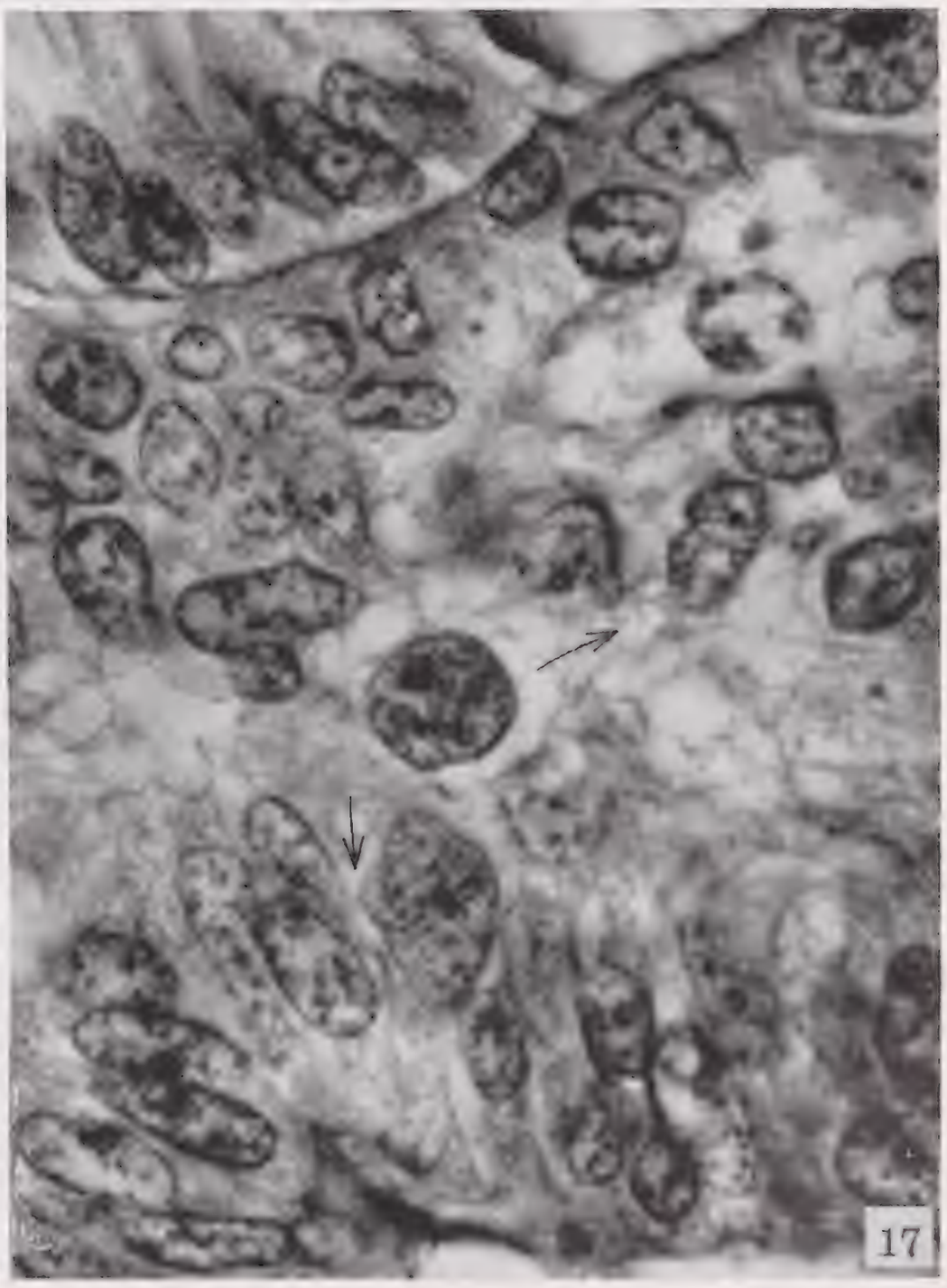
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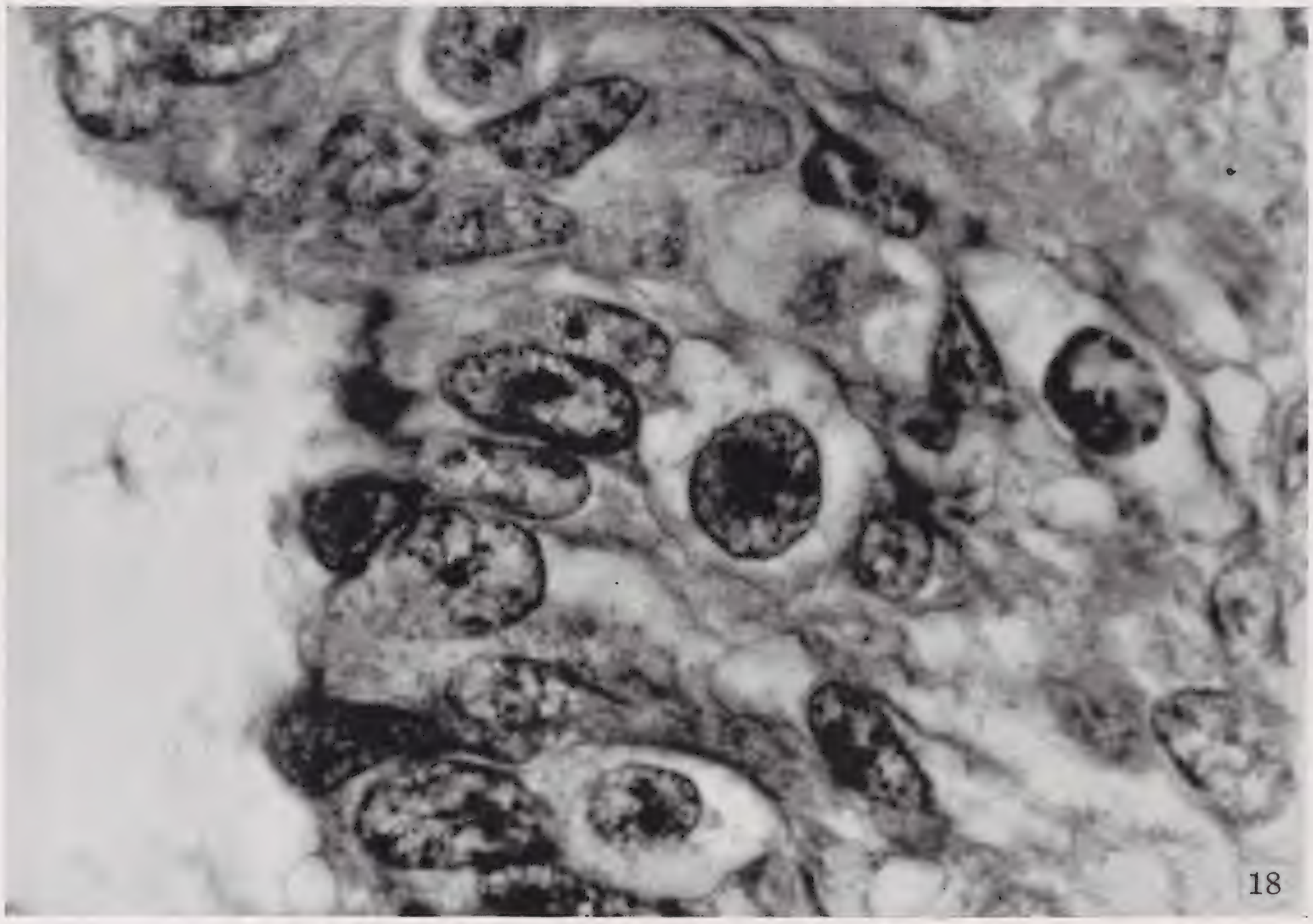
## Plate 6

Embryo no. 7889, 32 somites, 4.2 mm.; sections 6 microns

Figs. 18, 19. Amoeboid germ cells moving along the peritoneum of the left coelomic angle. Sections 3-2-6, 3-2-14. ×2000.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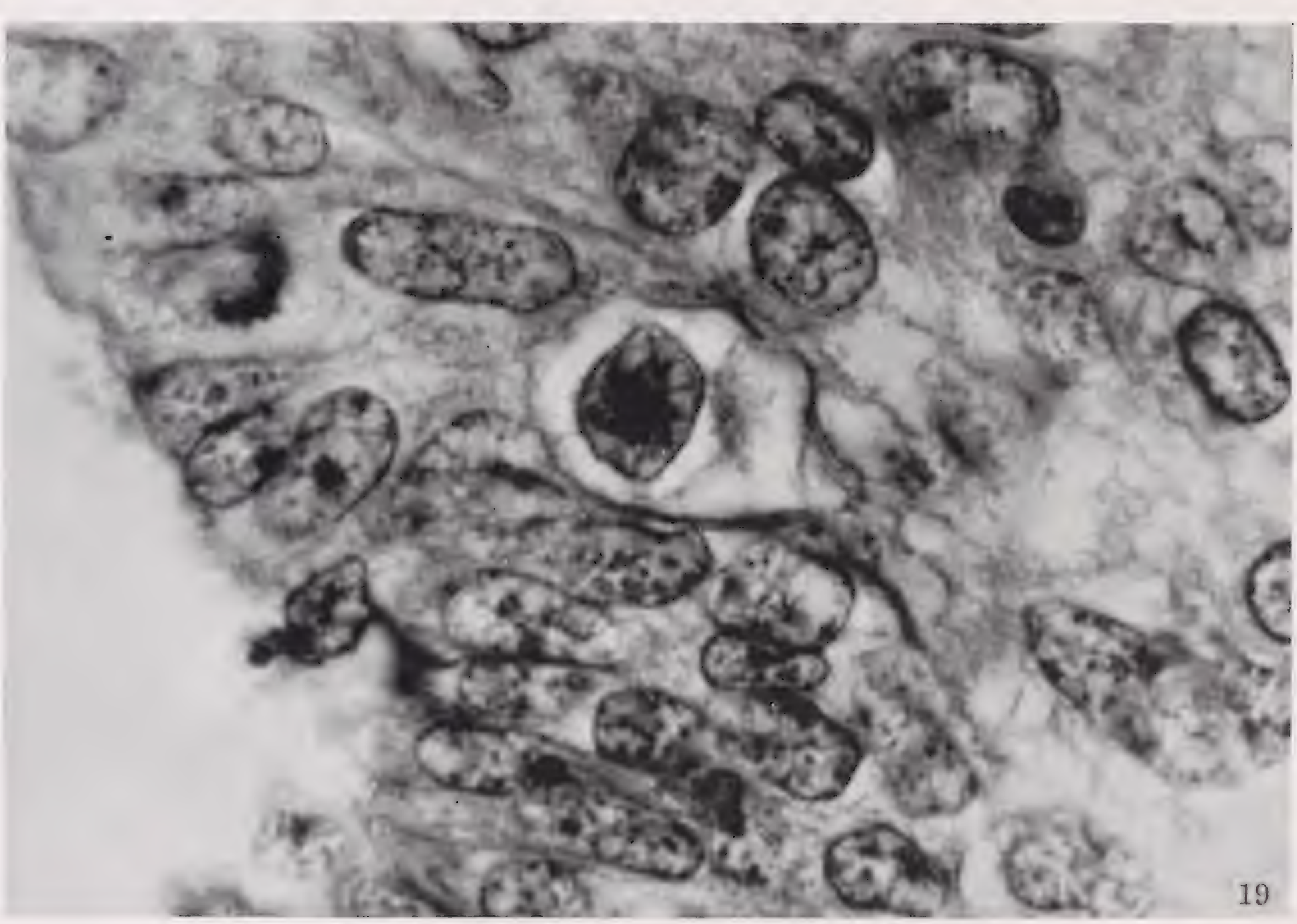
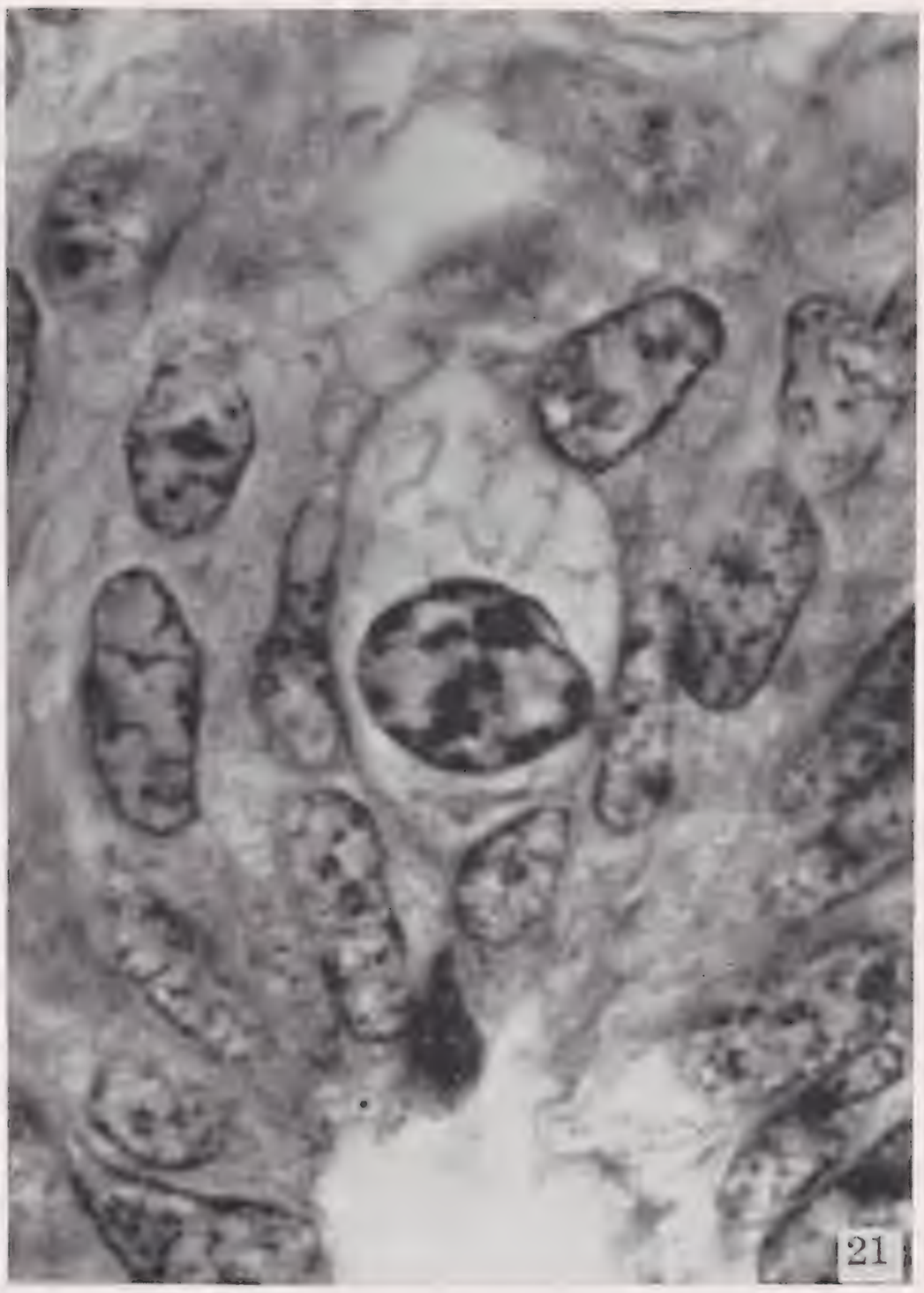

19

## Plate 7

Embryo no. 7889, 32 somites, 4.2 mm.; sections 6 microns

Figs. 20–23. Four germ cells in the mesentery or at the coelomic angle, at various stages of entering or leaving the peritoneal epithelium (see text). Sections 3-3-3, 4-1-13, 3-4-11, and 4-1-6. ×2000.

20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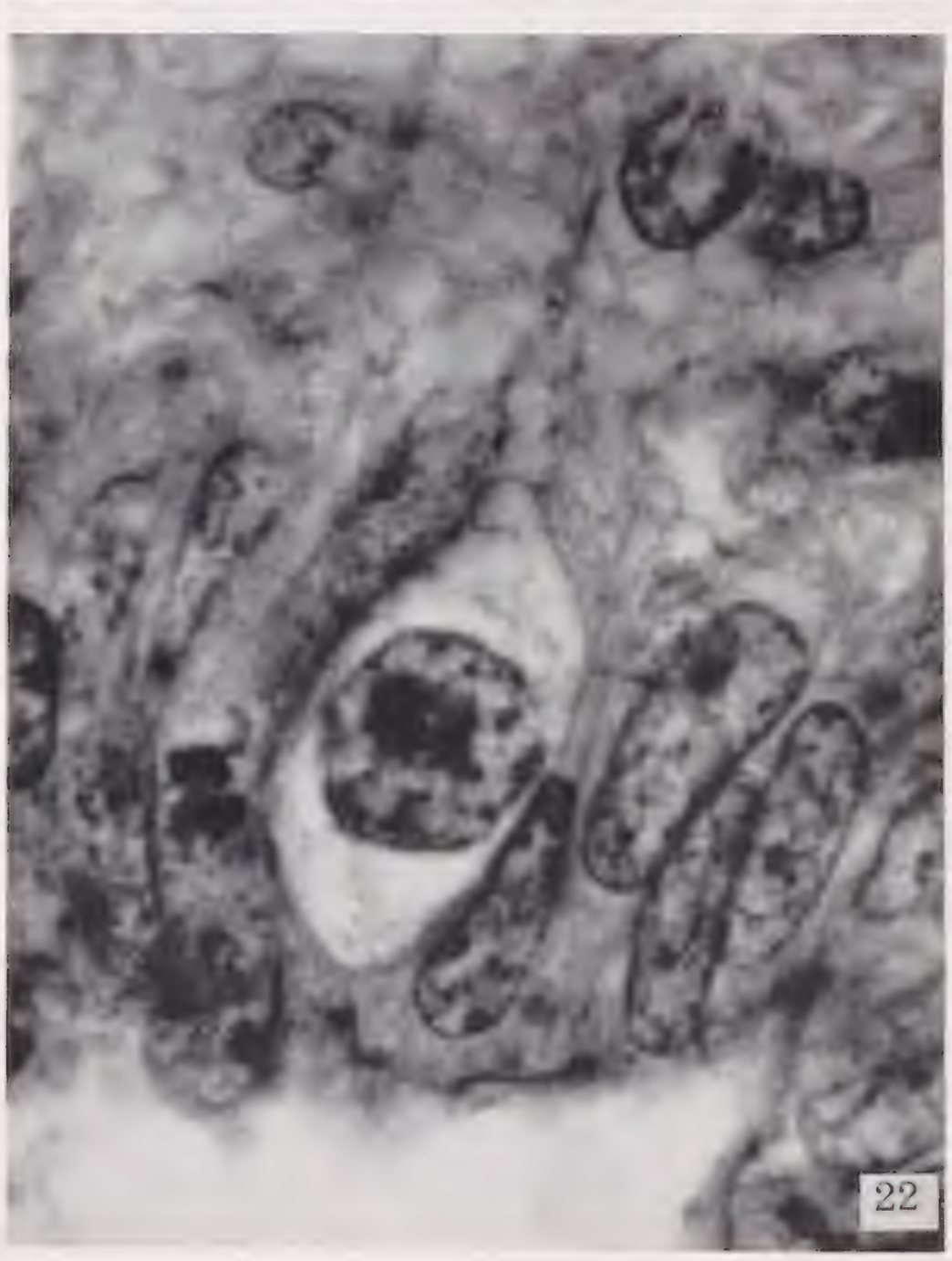
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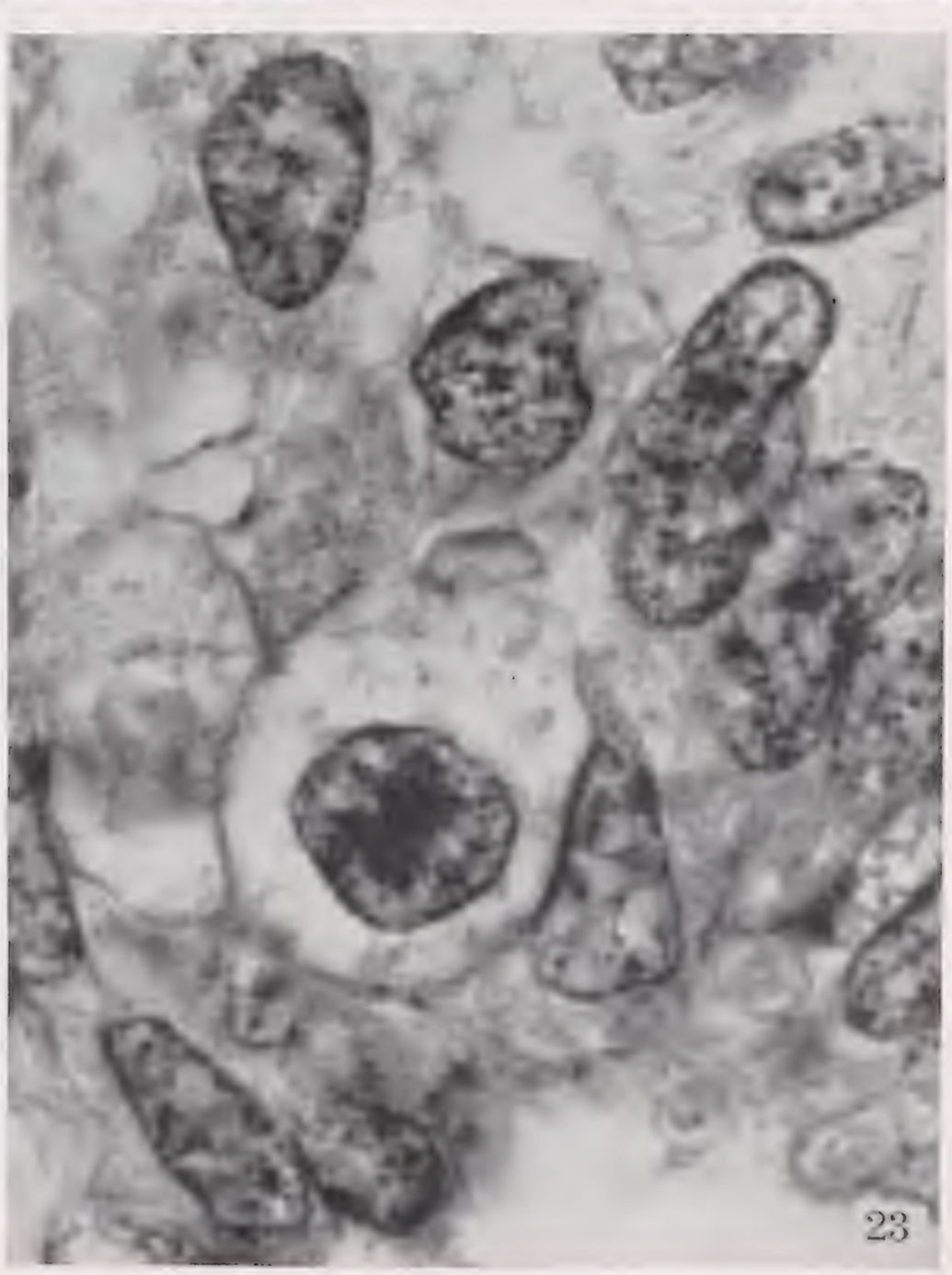

23

## Plate 8

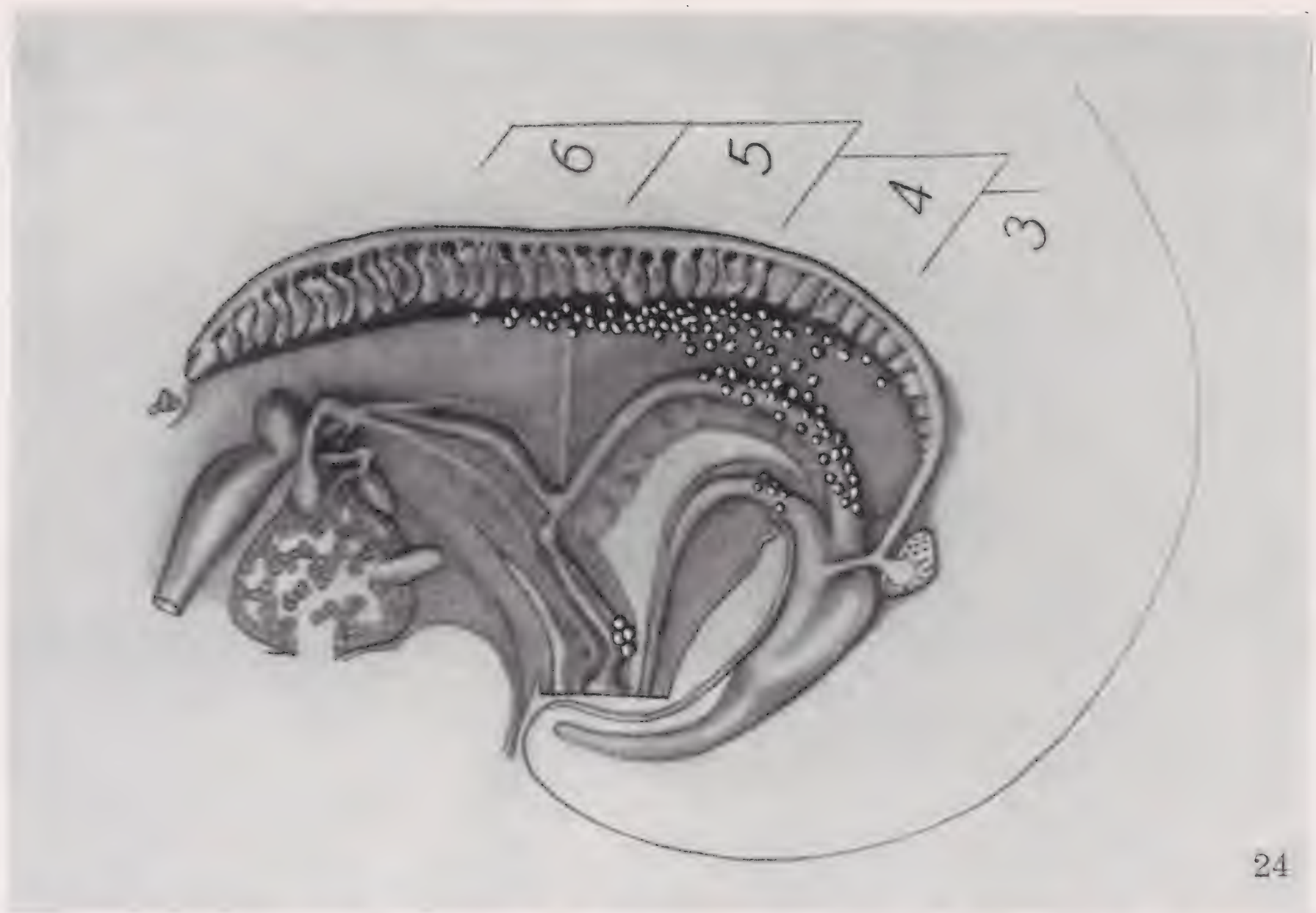
Embryo no. 1380, 38 somites, 5.5 mm.; sections 20 microns

Fig. 24. Graphic reconstruction of the nephric and endodermal organs, with their mesenteries, of the lower part of the embryo. The plane of micro-sectioning and the levels of the third to sixth slides are indicated (compare with table 3). The migrating germ cells are represented as if shining through their covering tissues; their size is somewhat exaggerated. Two clusters of extra-regional gonia are present in this embryo: one at the base of the allantoic stalk, the other in the umbilical part of the yolk stalk. ×40.

Fig. 25. Germ cells at the dorsal crest of the hind-gut. Section 4-1-5. ×500.

Fig. 26. Germ cells in the yolk stalk. Section 4-1-5. ×500.

Fig. 27. Germ cells in the allantoic stalk. Section 3-4-1. ×500.

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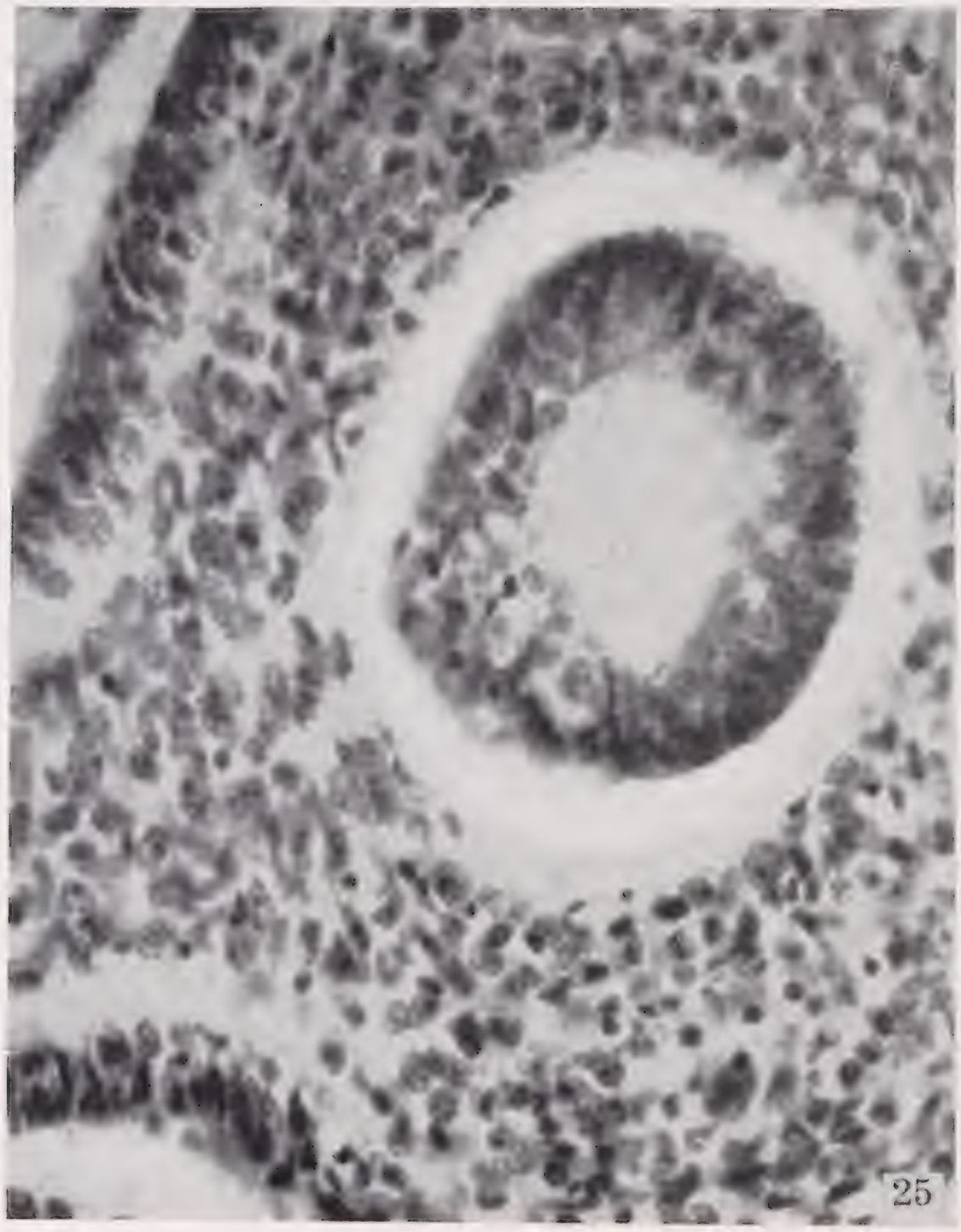

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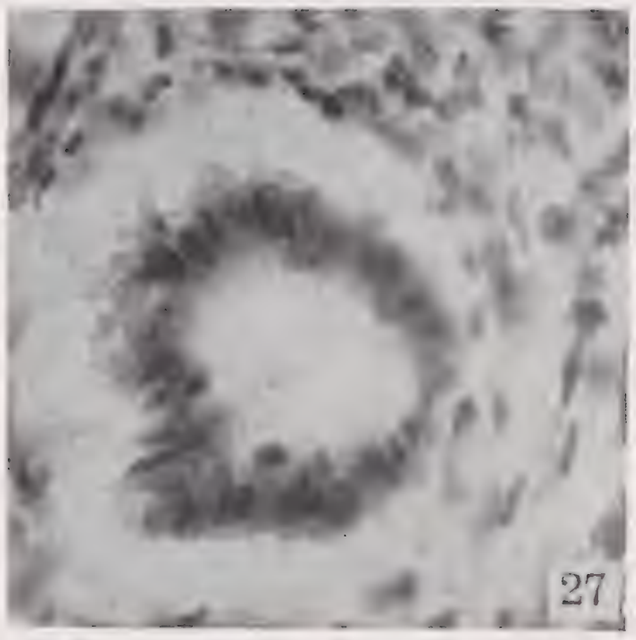

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26

Embryo no. 6513, about 7.4 mm.; sections 10 microns

FIG. 28. Cross section through left urogenital folds. In the gonadal region the cortical (peritoneal) and the medullary (mesonephric blastema) parts are distinctly separated by the thin tunica albuginea. The germ cells are either in the cortex or in the albuginea. Section 11-1-5. ×300.

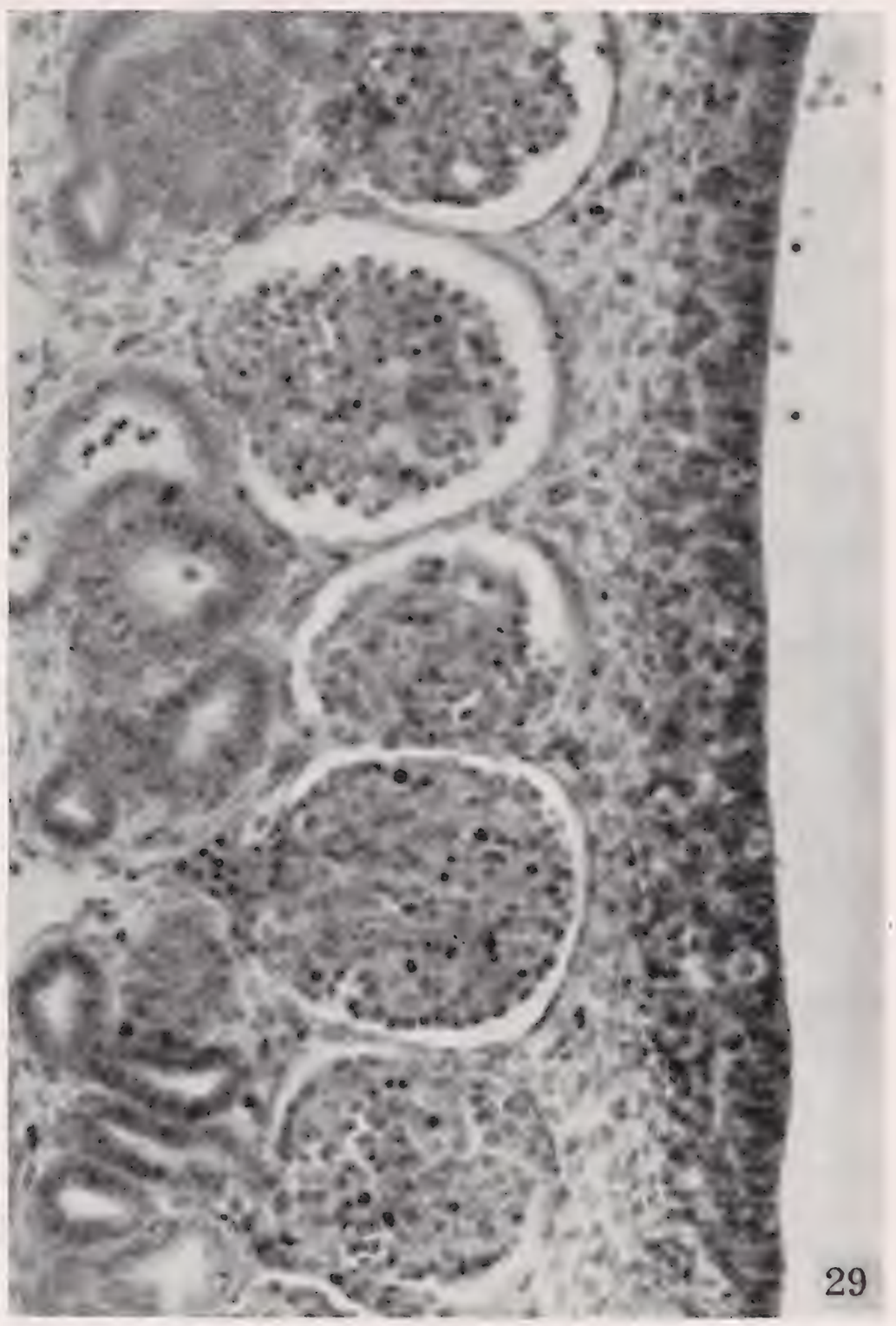
Embryo no. 6511, about 8 mm.; sections 10 microns

FIG. 29. Longitudinal section through part of a urogenital fold. As in figure 28, the peritoneal and the blastemic components are separated by a fine tunica albuginea. Most germ cells are in the cortex, but some have become transferred to the medulla. Section 6-4-4. ×300.

Embryo no. 792, 8 mm.

FIG. 30. Graphic reconstruction of the urogenital and lower endodermal organs with their mesenteries. The distribution of the germ cells in cortex, albuginea, and medulla along the lower half of the mesonephros is indicated by white globules. Their size is increased, their number reduced in comparison with the actual condition. ×50.

28



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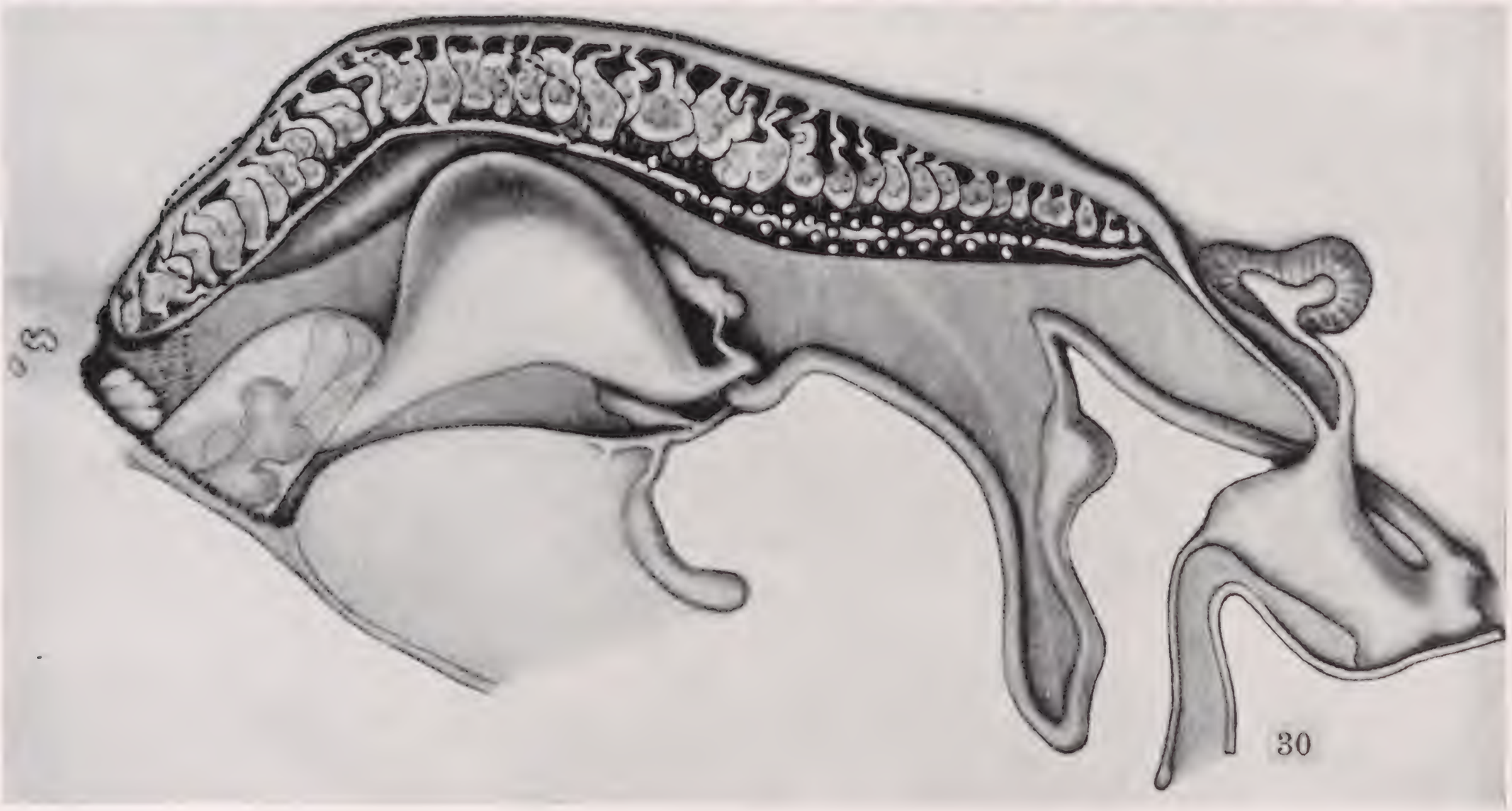

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CONTRIBUTIONS TO EMBRYOLOGY, NO. 210

# THE DEVELOPMENT OF THE GONADS IN MAN, WITH A CONSIDERATION OF THE ROLE OF FETAL ENDOCRINES AND THE HISTOGENESIS OF OVARIAN TUMORS

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With six plates

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# THE DEVELOPMENT OF THE GONADS IN MAN, WITH A CONSIDERATION OF THE ROLE OF FETAL ENDOCRINES AND THE HISTOGENESIS OF OVARIAN TUMORS

## INTRODUCTION

This study of the development of the human ovary and testis was intended originally as a preamble to a much larger work on the histogenesis of ovarian tumors. The present unsatisfactory status of that subject and the frequent inability of pathologists to explain, on the one hand, the complex histological pictures encountered in hormone-producing tumors, and, on the other, the physiological effects of such tumors, seem due in no small measure to a lack of understanding of the origin and the potentialities of the various cell types in the ovary as well as to a failure to recognize the dynamic relations of the different cells at various phases in their life cycle.

The histological pattern of the human gonad is determined very early in development, and it is difficult in later stages to establish with certainty the origin of any cell in the ovary or testis. Any such attempt in postnatal ovaries can be very misleading, because of the extraordinary cytological transformations which have occurred. Some of the most crucial phases occur in the period between the first thickening of cells in the genital ridge and the time when sex is recognizable histologically. During this relatively short period the primitive sex cells and the precursors of most of the other constituents of the gonad first become associated. The rapidity of this process and the difficulty of following the movements of the various cellular elements, derived from different sources, as well as the problem of establishing reliable criteria for identifying these cells in the relatively formless gonad, have left ample opportunity for speculation and controversy. The literature on this subject is a testimony to the confusion which can arise when basic problems of a strictly morphological character remain unsolved. There is scarcely an aspect of early gonadal development on which investigators are unanimous. A sharp division of opinion exists even on such a fundamental issue as the role of the coelomic epithelium in gonadogenesis. This is not a matter of theoretical interest alone, since the interpretation of ovarian tumors largely hinges on the satisfactory solution of this question.

If the coelomic epithelium contributes nothing to the ovary except its epithelial covering, as Fischel (1930) claims, then clearly the theca cell and the granulosa cell are derived from a common stem cell. On this basis it would be anticipated that in tumors derived from the stem cell, both theca and granulosa cells would be found. Traut and Marchetti (1940) have found that in a large number of granulosa cell tumors, theca cells are present to a greater or less extent. This finding is used quite naturally to substantiate Fischel's hypothesis. On the other hand, if the coelomic epithelium does participate in gonadogenesis, and if, as will be indicated in this study, it forms only granulosa cells, then on what basis is it possible to account for the association of theca cells and granulosa cells in the same tumor? Is this association due to a metaplasia of one type into the other, or has it some other underlying cause which can be exposed by embryological studies? Acceptance of a hypothesis merely because it circumvents a dilemma is misleading and may obscure the facts. These considerations reopen the whole question of the potentiality, independence, and interdependence of the cell types in the human gonad, which is the main theme of the present study.

The developmental problems of the sex glands are of perennial interest because of the curious aberrations which find expression not infrequently in such conditions as sex inversion and intersexuality, which are almost impossible to understand without a knowledge of the physiological processes involved in the differentiation of the gonads and the emergence of sex. Moreover, a study of gonadogenesis in both sexes should facilitate an understanding of the problems of arrhenoblastomata and other masculinizing tumors of the ovary. Though it is not possible to enter into this field with abandon, some attempt will be made to indicate the interrelationship of the more important endocrine glands, the gonads, and congenital abnormalities of the sex organs. It is customary to accept the fact that maternal hormones in part influence the development of the gonads of both sexes, but

more especially of the ovaries. This is implicit in the corrected statistics which Hunter (1930) submitted to account for the rapid growth of the fetal ovaries and uterus toward the end of gestation. Such a view, as will be shown, is not entirely justified, as it does not take into consideration the extraordinary hyperplasia and hypertrophy of the interstitial cells of the testis and their atrophy toward the latter half of pregnancy. Although it is not possible to deny this view categorically, at least we may expose the discrepancies which make it unacceptable in its present form.

This study is divided into three parts. The first is descriptive, and the second is devoted to a critical analysis of the theories connected with the origin of the germ cells and the other constituent elements of the gonads. The life history and the potentialities of the granulosa and theca cells have been closely examined with reference to the value of these findings in the interpretation of some types of ovarian tumors. Ovary and testis have been considered comparatively in order to provide a factual background for the understanding of sex inversion and hermaphroditism. The concluding section is devoted to physiological considerations affecting the growth and differentiation of the human sex glands.

It has not been possible to deal with the extensive literature in the manner originally planned, because of the unsettled circumstances under which the study was conducted and the inability to make plans for more than a few days at a time. It was not possible to take full advantage of the library facilities at the Johns Hopkins Medical School, and in South Africa such facilities are very limited. It seemed advisable at the time to take full advantage of the unique material in the Carnegie collection rather than to deal exhaustively with the literature. Fortunately, sufficient time was available to read again the classic works of Waldeyer, Kölliker, and others, cited frequently hereafter.

## MATERIAL

The material consisted of 250 specimens derived from three sources: the Carnegie collection, the human ovaries in Dr. T. R. Forbes' collection, and ovaries and testes of older fetuses prepared in the Department of Anatomy of the University of the Witwatersrand. The serially sectioned embryos belonged exclusively to the Carnegie collection. Thirty of these measured between 3 mm. and 9.9 mm., 38 between 10 and 19.9 mm., 19 between 20 and 29.9 mm., 10 between 40 and 60 mm. All the ovaries listed by Dr. Forbes in his paper of 1942 were also examined. The fetuses were from 100 mm. up to term, and 11 ovaries were from newborn infants and children up to 3 years of age.

## THE EARLY DEVELOPMENT OF THE GONAD

The genital primordium is first discernible when the human embryo has a crown-rump measurement of 5.5 to 7.5 mm. Prior to this there is no visible evidence of gonad development, although many other organs are well advanced. The liver cords are present, the pronephros has formed, the thyroid is a stalked sac, the mesonephric tubules are actively differentiating, and Rathke's pouch is already prominent. The suprarenal invariably differentiates from the coelomic epithelium in the dorsomedial angle of the body cavity before there is any indication of the genital primordium. In fact, at the time when the suprarenal forms, the epithelium covering the mesonephric bulge is multilayered, like that covering the root of the mesentery; but this condition is not identical with the thickening which occurs in the genital ridge at the time when the gonad begins to differentiate.

The gonad has at least a double, and if we include the germ cells a triple, origin. The two main constituents are represented by the coelomic epithelium of the genital ridge and the underlying mesenchyme. Both primordia may differentiate simultaneously or one may precede the other. The coelomic epithelium involved covers the mesonephric bulge from the dorsomedial coelomic angle downward to the ventral surface of the mesonephros. In a craniocaudal direction the genital epithelium is at first more or less coextensive with the mesonephros; later it is restricted to its intermediate two-thirds. The mesenchymal primordium is represented by a concentration of cells

deep to the genital ridge and coextensive with it. This primordium is depicted in figure 1 of Stieve (1930) and is the one which impressed Fischel (1930) so much that he thought the whole gonad was derived therefrom.

In figure 1, plate 1, which is a transverse section of an embryo of 6.7 mm. C.-R.L., both parts are already present. The coelomic epithelium consists of a mass of oval or ellipsoidal cells crowded closely together. They are not separated from the mesenchyme by any structure which could be called a basement membrane, and are so crowded that no cell boundaries can be identified. The nuclei are arranged in different levels, giving the epithelium an irregular appearance, whereas in other regions it is more regular and the nuclei are more vesicular. Almost the entire cell is filled by the nucleus. Although the cells covering the genital ridge are actively proliferating and closely crowded, they have not invaded the underlying mesenchyme.

Apart from the usual reactions preliminary to mitosis, the cells of the thickened epithelium are cytologically indistinguishable. No cells are present which can be regarded as primitive sex cells, as seems to be the case in the rat (Hargitt, 1925), or in some reptiles (Berenberg-Gossler, 1914). In fact, at no stage of the human gonad are typical sex cells present in the active coelomic epithelium, as is the case in the chick, where they enter it secondarily (Swift, 1914). If they ever enter the coelomic epithelium in human embryos, then they lose all identifying characteristics.

Deep to the thickened genital epithelium the mesenchymal primordium is clearly evident in figure 1. The cells are concentrated into a compact mass. Not only are they more crowded than in adjacent regions, but their long axes lie at right angles to the coelomic epithelium, whereas in other regions they are parallel to it. The cells in this mesenchymal primordium frequently assume a cordlike structure, an appearance which apparently confused Fischel (1930). They are separated from the mesenchymal cells of the mesonephric body by an area in which cells are not very numerous, as is seen particularly well in figure 1.

Even at this early stage of development a few large, clear cells are present amid the compact layer of mesenchyme. In figure 1, two are seen, and one appears in figure 2. Similar large cells were seen also by Felix (1912), who named them genitaloid cells. Felix thought that they degenerated and that the true sex cells were formed from the genital epithelium. Stieve (1927) portrays similar cells in a 4.7-mm. embryo in his figure 2, and Kohno (1925) described "Urgenitalzellen" in the lateral plate mesoderm in a 2.3-mm. human embryo. Similar cells are specifically identified by Fuss (1912) in the intestinal epithelium of a 4-week-old human embryo. He suggested that they migrate from the gut into the gonad region. In anuran amphibia, most investigators are agreed that the primitive germ cells arise in the dorsal wall of the gut endoderm, the so-called "yolk ridge" (Willier, 1937, 1939); Bounoure (1934, 1936) traced them all the way back to cells which are set aside during early cleavage. In the horned toad (Jarvis, 1908) and in the turtle (Allen, 1906; Risley, 1933*a*, 1933*b*), the germ cells are said to arise in the endoderm and later, by migration, to enter the genital primordium.

Simkins (1928) states emphatically that in human embryos he was unable to discover "cells of peculiar size or staining reaction anywhere in the body of embryos as young as the first month." He concluded that strict independence of somatic and germ cells is not demonstrable in the history of the human gonad. As will be shown later in this description, the primitive sex cells multiply rapidly and are a characteristic feature of the gonads of older embryos (figs. 2–6, pl. 1). It is for this reason that the view is taken here that the human gonad has a triple origin: from the coelomic epithelium covering the genital ridge, from the underlying mesenchyme, and from primitive sex cells, probably derived early in development from another region.

A later stage in development is depicted in figure 2, a frontal section through the genital ridge of an embryo 8.2 mm. C.-R.L. The genital primordium is still not demarcated from the rest of the mesonephric body, but the coelomic epithelium of the gonadal area is very much thickened. The cells are proliferating actively, accumulating locally to form short buds, the sex cords, which invade the underlying mesenchymal primordium. The radial arrangement of the mesenchyme, a prominent feature in figure 1, has apparently facilitated the ingrowth of the cords from the coelomic epithelium. The interdigitation of the sex cords and the cells of the compact layer of mesenchyme makes it increasingly difficult to discriminate between the two primordia. If embryos were examined at this

stage alone, it would be excusable to regard the dense mass of cells in the primordium as having arisen solely from the coelomic epithelium, but it would be inexcusable to maintain that the gonad is derived only from the mesenchyme, as Fischel (1930) thought.

The mesenchymal cells and those from the coelomic epithelium are cytologically very similar. The nuclei are relatively large and moderately chromatic, with one or two prominent nucleoli; and the cytoplasm is so reduced that the cords seem to consist of a mass of nuclei in a syncytium. Close examination, however, reveals that the cells of the cords are different from those of the mesenchyme. Considering also their appearance in earlier stages of development, it is obvious that the mesenchymal primordium is still a noticeable feature of the gonad at this stage of development, albeit the sex cords have broken up the compact mass into a number of separate segments, which are still continuous beyond the free extremities of the cords. The older the embryo, the more difficult it becomes to distinguish these two components of the gonad.

In figure 2, several primitive sex cells are to be seen. One is particularly well shown (*psc*), lying well below the coelomic epithelium. It cannot be confused with a developing blood cell, nor can it be regarded as a dividing cell, either from the coelomic epithelium or from the mesenchyme. This primitive sex cell is at least twice as large as any other cell in the vicinity; its oval cell body is well defined, and the large vesicular nucleus contains a prominent nucleolus. The sex cords seem to curve around it. The primitive sex cells are always situated well below the surface throughout the early period of development. Such cells are not encountered at all in the coelomic epithelium.

A sagittal section through the caudal half of the gonad in an embryo of 9.6 mm. C.-R.L. (fig. 3) reveals considerable progress. The coelomic epithelium is much thickened, mitotic figures are numerous, and the sex cords have penetrated the whole thickness of the compact layer of mesenchyme, thus coming into contact with the general mesenchyme of the mesonephros. In the cranial region, where the sex cords are in contact with the general mesenchyme, they are beginning to bend at right angles to their long axis. This is significant, since it foreshadows the appearance of the rete complex, derived apparently from the deepest part of the sex cord. Mitotic figures are not common in the sex cords themselves, though abundant in the outer cellular region of the gonad.

The sex cords begin to develop, therefore, in embryos between 6.7 mm. and 8.7 mm. C.-R.L., lengthening by an accession of cells from the coelomic epithelium. The original single layer of coelomic cells has differentiated into two distinct parts, namely, a compact, thickened epithelium which covers the gonad, and the cords which are derived therefrom.

The invasion of the underlying tissue by the sex cords has now broken up the mesenchymal primordium into loose strands interdigitating with the cords. The individual cells remain small, but are widely spaced. The relatively dense sex cords alternate, therefore, with less dense regions comprising the mesenchyme and the primitive sex cells (fig. 3). Despite this intimate interrelation, the cells of the cords can still be distinguished from the mesenchyme, although not as readily as in a younger embryo. The primitive sex cells have increased in number, and since they lie between the cords they accentuate the general loosening of the mesenchyme. The sex cells increase in proportion to the lengthening of the sex cords (figs. 5, 6). In a high-power view of a detail of figure 3, a finer analysis of the developing gonad is possible (fig. 4). The irregular arrangement of the cells of the thickened coelomic epithelium can be observed. The round or oval nuclei are moderately chromatic and contain one or two prominent nucleoli. Cell walls are difficult to see. One mitotic figure is easily identified. As the sex cords invade the mesenchyme, the nuclei of their constituent cells become progressively smaller.

A notable feature of figure 4 is the primitive sex cells. Their nuclei are very large, round or oval, and vesicular, with two or three prominent nucleoli. Finely divided chromatin is scattered evenly throughout the nucleus. No cells of this type can be identified in the coelomic epithelium, nor are there any forms transitional between the two types. If the primitive sex cells were derived from the coelomic epithelium, it would be reasonable to expect transitional forms, or at least some cells in the surface epithelium from which the primitive sex cells could have been derived; but the primitive sex cells, as we have seen, appear in the mesenchymal primordium even before the coelomic epithelium has proliferated, and there is no sign that the primitive sex cells are being incorporated into the early sex cords. This phenomenon is not encountered

until later, when there has been a considerable lengthening of the cords.

Once the sex cords have begun their penetration of the mesenchymal primordium, development is rapid although it varies in different embryos of the same size. In a 10.6-mm. embryo (fig. 5) the gonad is comparatively enormous, projecting far into the peritoneal cavity. It is still attached to the mesonephros by a broad base, but already deep grooves indicate the formation of the mesorchium (or mesovarium). Growth of the gonad proceeds centrifugally. This fact accounts for the limited penetration of the sex cords in the direction of the mesonephros.

The main features of the gonad at this stage are the broad base, the outer cellular layer, the length and complexity of the sex cords which (at their central ends) form the rete complex, the large numbers of primitive sex cells between the sex cords (more abundant near the periphery but scarcely recognizable in the central and basal regions of the gonad), the breaking up of the mesenchymal primordium, and the vascularization of the sex gland, by way of its basal attachment to the mesonephros. Although sex is still indeterminate, the gonad resembles a testis more than an ovary, a distinctive characteristic of all sex glands at this stage. The resemblance to a testis consists in the complexity of the sex cords, which are distinctly separated by loose mesenchymal tissue. On the other hand, the thickened coelomic epithelium and the abundance of primitive sex cells immediately beneath it are features reminiscent of the primitive ovary. Indeed, the gonad depicted in figure 5 is sexually bipotential.

Closer examination shows that the sex cords are much folded as they extend toward the root of the gonad. In their outer third they are clearly separated and retain their continuity with the epithelial covering of the sex gland. In this zone mitotic figures are numerous, whereas they are singularly lacking elsewhere. Growth is apparently maintained through the activity of the coelomic epithelium, and the gonad grows chiefly by accretion in a centrifugal direction. In the intermediate zone the cords lie closer together and are less easily recognized, owing to the reduction of the mesenchyme and the absence of the large primitive sex cells. Toward the attached border of the gonad the cords are more compact and the nuclei more chromatic than in the peripheral segments. The free extremities of the sex cords represent the primitive rete complex. The rete complex therefore is a derivative of the coelomic epithelium, the oldest part of the sex cord, and the first part to be formed when the epithelium begins to invade the mesenchyme.

The primitive sex cells are numerous and easily seen in the outer third of the gonad. In the deeper regions they are not easily recognized at low magnifications, since they are smaller, because of multiplication, and many are already included in the cords. At the base of the gonad the mesonephric mesenchyme is denser than in younger embryos. Small vessels enter the gonad, and mesenchymal cells become rearranged to form sheaths about them as they extend between the sex cords. In figure 5 the hyperchromatic nuclei of the rete cords indicate the line of demarcation between gonad mesenchyme and that of the mesonephros.

At higher magnification (fig. 6) it can be seen that the coelomic epithelium consists of three or four layers of cells, closely packed, and much smaller than in younger embryos (compare fig. 4). Cell walls are very indistinct. At intervals the sex cords arise from the inner aspect of the epithelium, and in some instances they fuse to form relatively thick cords. The gonad has a looser structure just below the coelomic epithelium than elsewhere. Cell walls are obscure, so that the form of the cord is defined by linear aggregations of nuclei.

The primitive sex cells lying between the cords are very large and apparently more numerous than elsewhere in the gonad (fig. 6). Some have well defined cell membranes, although the cytoplasm is clear save for a concentrated mass near one pole of the nucleus, which probably represents the Golgi apparatus and centrioles. The sex cells now vary considerably in size and appearance. At *psc 1* is a large cell like that shown in figure 4; near by is a much smaller cell otherwise resembling its large neighbor. Other smaller cells (*psc 2, psc 3*) are found in intimate contact with sex cords. Deeper in the gonad, where the cords are closely packed, primitive sex cells can be distinguished only with difficulty and seem to be incorporated into the cords. For a while at least, they lose their conspicuous character.

The primitive sex cells do not become associated with the sex cords until some time after the latter have evolved; so that the earliest part of the cord contains

but few sex cells, whereas the more recently formed segment is more richly supplied. Those parts of the cord which will form the rete and the straight segment of the seminiferous tubule contain only an occasional sex cell, and are formed almost exclusively of cells derived from the coelomic epithelium.

Because the sex cells in the male diminish in size and are incorporated early into the sex cord, their fate is difficult to follow; but in the female, where they persist unchanged for several months and then enlarge to form oögonia, their fate is not so open to question. There is no valid reason to suppose, as did Felix, that the primitive sex cells atrophy and disappear, and that definitive sex cells arise anew from the coelomic epithelium. It would be strange indeed if in the male (in which they are numerous at the time of formation of the sex cords) they should mysteriously vanish after incorporation into the cords, whereas in the female they clearly persist for a much longer period and give rise to the oögonia, as will be shown. It is for these reasons that the large cells seen below the coelomic epithelium of the genital ridge in very young embryos are regarded as the primitive sex cells, the precursors of the true germ cells.

The mesenchymal part of the genital primordium is first utilized in providing a sheath for the sex cords. It first appears as small, spindle-shaped cells which can be seen most clearly in the rete region. In figure 6, however, some of these cells can be seen at *mes*. They differ completely from the primitive sex cells and the cells of the cords. This primitive connective tissue is later reinforced by an accession of cells from the mesenchyme in the base of the gonad, in association with the ingrowing vessels. In the 10.6-mm. embryo there is already evidence of this secondary ingrowth, and in figures 6 and 7 (the latter a high-power view of the basal region) capillaries can be seen to have invaded the gland. Their position is indicated by the nucleated blood cells shown at *bc* (fig. 7). Surrounding these capillaries are mesenchymal cells which are intruding between the rete cords.

Just before sex can be recognized histologically, differentiation appears to predominate over growth. The thickened coelomic epithelium is still very irregular, but the individual cells are not so small as in figure 6, and cell membranes are more distinct than in earlier stages. The outermost layer is separated from the sex cords by a thick cellular zone containing numerous primitive sex cells; scattered, spindle-shaped mesenchymal cells; and numerous cells derived from the coelomic epithelium, indistinguishable from those of the cords. In the female, this thick cellular zone grows steadily, and Felix regarded it as forming the ovarian cortex. He named it, accordingly, the neogenic zone. This term is a misnomer, since it implies that the ovarian cortex is formed exclusively from the neogenic region, a view which is untenable. The cellular layer, or zone, is not peculiar to the ovary. It is present in the indifferent gonad, and in the testis undergoes a spectacular transformation to form the tunica albuginea (figs. 9, 12, pl. 2).

It is stated that in the male chick the coelomic epithelium proliferates once to form the primary sex cords, whereas in the female two separate proliferations occur, the second of which gives rise to the ovarian cortex, while the primary sex cords become the medullary cords, homologues of the seminiferous tubules (Willier, 1939, and others). The broad cellular zone in the chick is therefore regarded as a female characteristic, although Willier adds that in both sexes the embryonic gonad has bisexual potentialities.

In the human embryo it is not the thickness of the cellular layer which is a criterion of sexual differentiation, but rather the nature of the metamorphosis of its constituent cells. In the ovary the germ cells increase in number and the smaller cells, derived from the coelomic epithelium, also enlarge. In the male both types of cell diminish rapidly in size and become dispersed in an edematous matrix; the germ cells disappear, and the cellular zone is transformed into the tunica albuginea. The thickness of the outer cellular layer therefore is not alone a reliable criterion of sex.

The sex cords occur as irregular strands, continuous with the inner aspect of the cellular layer. Toward the hilum, however, they converge to form the rete complex. Stieve (1927) holds that the coelomic epithelium proliferates to form a diffuse mass of cells from which later the sex cords become suddenly organized. It has been shown here (figs. 2, 4) that the sex cords first arise as buds from the coelomic epithelium. The first parts to appear are precursors of the rete complex, and the last-formed segments become, in the male, the seminiferous tubules. The rete cords are ensheathed at an early stage by cells derived from

the mesenchymal primordium, but peripherally this sheath is less evident, so that in the indifferent gonad the sex cords are closely packed together and the cells of the cord remain broadly continuous with the cellular zone (fig. 8). Mitotic activity is at a low ebb. The fetal connective tissue is sparse toward the periphery. In some tangential sections it appears that the sex cords are separated from the cellular zone by a layer of connective tissue, regarded by some as the primary tunica albuginea. In serial sections it is clear that this connective tissue does not form a continuous layer. The so-called primary tunica albuginea is suggested by the way the sheath material stops suddenly where the cords blend with the cellular layer. The sex cords and the cellular layer remain continuous in the male until the cellular layer differentiates into the tunica albuginea (fig. 9); whereas in the female, the septa extend through the cellular mass to spread out below the outermost layer of the coelomic epithelium. There is thus no clear separation between the so-called primary sex cords and the outer cellular layer in the ovary.

In the cellular zone, primitive sex cells are still recognizable by their size and characteristically large nuclei, but in the sex cords they are difficult to identify, coming to resemble, superficially at least, the other cells in the cord. Should the indifferent gonad differentiate into an ovary, however, they enlarge to form oögonia, and can then be seen throughout the gonad, although much more numerous in the cortical region (fig. 20, pl. 4). Not infrequently oöcytes are included in the tubules of the rete ovarii, but these sooner or later perish.

When differentiation of the gonad sets in, it is much more evident in the testis than in the ovary. In the male, the cords become markedly condensed to form seminiferous tubules, and the tunica albuginea develops from the thick cellular layer. The rete cords are more prominent, and in general the young testis assumes, in miniature, the structure of the fully formed gland. In the ovary, differentiation is much less spectacular; the rete cords are smaller, the intercordal tissue is sparser, and the sex cords are consequently less defined. The outer cellular layer, with its numerous sex cells and other elements, remains active. Actually the gonad is an ovary, not because it has that structure, but rather because it is not a testis. The ovary only begins to resemble the adult organ late in development (12th to 16th week), when the fetus has a crown-rump measurement of 120 to 140 mm. The testis thus develops precociously, whereas the development of the ovary is comparatively delayed.

To summarize at this point, the human gonad has a triple origin, namely, from the coelomic epithelium of the genital ridge, from the underlying matrix of mesenchyme, and from the primitive sex cells, which reach the mesenchymal primordium from some other region. The coelomic epithelium gives rise to the sex cords, the first buds of which form the rete complex; the germ cells diminish in size and are taken into the sex cords. Continued proliferation of the coelomic epithelium forms a thick cellular layer, continuous with the sex cords, in the deeper regions of which primitive sex cells are later found in abundance. The primitive sex cells do not appear to enter the coelomic epithelium to form a germinal epithelium *sensu stricto*.

The mesenchymal primordium forms the supporting tissue of the sex cords and it later receives an accession of cells from the general mesenchyme of the mesonephric body. Capillaries invade the genital gland at an early stage. The sex cords remain in broad contact with the outer cellular zone, and there is no true primary tunica albuginea separating the sex cord from the cellular layer. As will be shown, the cellular layer in the male becomes the tunica albuginea, whereas in the female it persists as part of the fetal cortex.

## DIFFERENTIATION OF THE TESTIS

The differentiation of the indifferent gonad into a testis occurs typically in embryos with a crown-rump measurement of 14 to 16 mm. It manifests itself first in the great prominence of the seminiferous tubules (cords); second, in a decrease in the number and size of the cells of the cellular layer; and third, in the wide separation of the seminiferous tubules, or cords, by mesenchymal tissue. Even in young embryos the identity of the testis is unmistakable. In the differentiation of the testis the important elements are the tunica albuginea, the interstitial cells, the seminiferous tubules, and the rete testis.

## Tunica Albuginea

The tunica albuginea develops from the outer cellular layer of the indifferent gonad. A noticeable change is already apparent in a 15-mm. embryo. Sex cells seem to disappear or become so modified that their identification is difficult. The cells derived from the coelomic epithelium become smaller and are widely separated by an edematous matrix. It is not the thickness of the cellular layer that is distinctive in the male, as this varies from embryo to embryo of the same age; it is rather the loose structure and dispersal of the cells, and the disappearance of the primitive sex cells. Modification of the cellular layer is much more accentuated peripherally; near the hilum differentiation is slower.

For a time at least in the young testis the cellular layer retains continuity with the underlying cords, but in an embryo of 23.4 mm. some of the cords are separated from the young tunic by the intrusion of the mesenchymal sheath. In figure 8 the tunic consists of a thick layer of round or oval cells, continuous with the coelomic epithelium but now completely separated from the rounded extremities of the cords by the intervention of spindle-shaped cells. The coelomic epithelium is still actively adding to the growing tunica albuginea. Some cells of the tunic are reoriented so that their long axes are parallel to the surface. At a size of 34 to 38 mm. edema in the tunic is more pronounced, and the cells become more flattened and much more dispersed than in earlier stages. In figure 10, from a 54-mm. fetus, the reaction has involved almost the whole periphery of the testis to a point near the mesorchium. The distinction between the tunic and the region of the seminiferous tubules is now sharp (fig. 9). In a fetus of 132 mm. (fig. 12), the superficial epithelium is a flat layer like the mesothelium of the postnatal testis, and the spindle-shaped cells and collagenous fibers running parallel to the mesothelium foreshadow the final structure. There is now a sharp difference between the cells of the tunic and those lying between the seminiferous tubules, although the tissues are still continuous. This is especially well illustrated in figure 12, where the large interstitial cells contrast strikingly with the spindle-shaped cells of the tunic. Later when the interstitial cells atrophy, leaving large edematous spaces between the tubules (fig. 16, pl. 3), the intertubular tissue can still be easily distinguished from the fibrous tunic. In a 286-mm. fetus, the tunica albuginea is a thick layer of rather cellular fibrous tissue, covered by a flat mesothelium. Thereafter collagen fibers increase steadily until the tunic is like that of the fully developed testis.

The development here described reveals that the tunica albuginea is derived largely from the coelomic epithelium. At first the tunic layer (outer cellular layer) is continuous with the intertubular tissue, but gradually its cells differentiate into spindle-shaped fibroblasts. The history of the prospective interstitial tissue will follow. The tunic, however, also receives an accession of cells from mesenchymal elements which join it early, entering with the branches of the internal spermatic artery. Such vessels are prominent in the tunic of older fetuses (fig. 14, pl. 3).

Without having followed closely the evolution of the coelomic epithelium covering the genital ridge, it would be difficult to imagine that its cells could give rise to such diverse elements as the highly specialized sex cords on the one hand and the fibrous tunica albuginea on the other. The large series of embryos studied leaves, however, little doubt on this point. Even after the tunic is formed, the mesothelial layer continues to contribute cells which are converted into fibroblasts.

## Interstitial Cells

The interstitial cells of the human testis undergo a remarkable metamorphosis during development. From small spindle-shaped cells, lying between the tubules of the recently differentiated testis, they increase rapidly to form the extraordinary bed of cells seen in fetuses of 140 to 160 mm. The great volume of this bed, in proportion to that of the gonad as a whole, is not excelled even in postnatal life and maturity. After this initial hypertrophy a sudden atrophy supervenes, and the cells are reduced to small clusters of diminutive elements enveloped in loose connective tissue. At birth, interstitial cells comparable with those of fetuses of 100 to 140 mm. in length are uncommon.

It is extremely difficult to determine the origin of the interstitial cells once the gonad has become a testis. The various constituents of the gonadal primordium have been so transformed that it is no longer possible to state their origin without a knowledge of earlier stages. It has already been shown that the sex cords (exclusive of germinal elements) are derived from the coelomic epithelium, whereas the cells of the mesenchymal primordium pass in between the sex cords to form a supporting tissue. Before sexual differentiation, the compact, unbroken mesenchymal primordium ceases to exist as such, and its cells give rise to the interstitial cells as well as the sheath material. Some of these cells pass into the cellular layer (primitive tunic) and some merge with the general mesenchyme of the rete region.

As soon as the testis has differentiated (in 14- to 17-mm. embryos), the interstitial tissue is represented by spindle-shaped cells compressed between the sex cords. In 22-mm. embryos they are still very small and spindle-shaped, although more abundant than in younger embryos. In a high-power study of such a gonad (fig. 9) small interstitial cells predominate, but those near the tunic tend to be rounder, like similar cells in the tunic. In an older fetus, of 31 mm. C.-R.L., the interstitial cells show the first evidence of specialization by a sharper delineation of the individual cells. There is no marked change until the fetus measures 45 to 54 mm. Then they increase rapidly in size, owing chiefly to an enormous enlargement of the cell body. The cytoplasm is at first neutrophilic but in some cells tends toward basophilia. The nucleus is eccentrically situated, and at the opposite pole of the cell there is a crescentic mass of fine, basophilic granules.

Differentiation of interstitial cells occurs first at the periphery, immediately below the tunic, spreading radially and circumferentially from this region toward the junction of the straight tubules and rete. There is then a sharp distinction between true interstitial cells and the connective tissue of the rete region. In a fetus of 54 mm. C.-R.L. (fig. 10) the bulk of the testis consists of greatly enlarged interstitial cells, which contrast strikingly with the more solid seminiferous cords, the edematous tunica albuginea, and the compact connective tissue in the mediastinum testis.

Growth and differentiation of the interstitial cells proceed more rapidly than the growth of the seminiferous tubules. In a 113-mm. fetus the wide spaces are packed with enlarged interstitial cells. At 130 mm. C.-R.L., one-half to two-thirds of the testis (by volume) consists of interstitial cells, as may be observed in a low-power view (fig. 11) in which the interstitial cells appear as pale areas lying between the tubules. A section through the tunica albuginea and the subjacent zone in a 132-mm. fetus (fig. 12) shows the striking contrast between the young fibrous tissue and the large interstitial cells (compare fig. 9). In figure 9 the two tissues have in general the same morphological characteristics, whereas in figure 12 the interstitial cells are sharply differentiated from the spindle-shaped cells of the tunic. From now on the interstitial cells are arranged in cords or sheets, between which capillaries penetrate, as indicated by endothelial cells (fig. 13, pl. 3). In figures 12 and 13 the structure of the interstitial cell is well shown. In figure 12, *g*, there is a crescentic accumulation of granules which, in frozen sections, give a positive reaction for fat. These lipoids apparently contain a high percentage of protein, which remains in dehydrated sections.

The structure of large interstitial cells at this stage is very similar to that of the theca cells of atretic follicles in the ovary toward the end of gestation. The two types are not easily distinguished on the basis of cell morphology, and both resemble closely the cortical cells of the fetal adrenal before they accumulate fat.

The interstitial cells continue to increase in size until the fetus reaches a length of 160 to 190 mm. (fig. 14, pl. 3). The individual cell may be enormous, both cell body and nucleus being affected (fig. 15, pl. 3); the cytoplasm is very eosinophilic and riddled with small vacuoles, which give the cell a moth-eaten appearance. Not all vacuoles, however, represent dissolved fat globules. Some cells are very pale-staining as compared with the more eosinophilic cells, and these are already becoming atrophic—a process encountered in some fetuses of a much smaller size. The nuclei also vary in structure (figs. 13, 15). Some are moderately chromatic with a prominent nucleolus; others are vesicular, with the nucleolus even more highly accentuated. Still others have a wrinkled nuclear membrane which again heralds the beginning of atrophy.

By the time the fetus has reached a size of 286 mm. C.-R.L., the interstitial cells have become remarkably reduced both in size and in numbers (compare figs. 14

and 16). The intertubular tissue is now very loose and edematous (fig. 16). Here and there are small aggregations, mere remnants of the large masses of younger stages. The great reduction of the interstitial cells cannot be attributed simply to compression by the enlarging seminiferous tubules, for, as shown in figure 16, the tubules are still widely separated by edematous tissue of very loose structure. The interstitial tissue has also disappeared around the blind extremities of the seminiferous tubules, and a similar zone of loose tissue now intervenes between the tubules and the tunica albuginea (fig. 16).

This remarkable atrophy and degeneration of the interstitial cells is first expressed by a gradual loss of the eosinophilia, and the appearance of clear, non-staining areas throughout the cytoplasm. Vacuoles appear, enlarge, and fuse, displacing the nucleus to one pole of the cell. Sometimes the vacuoles surround the nucleus, which then lies at the center of a loose web, surrounded by a thin rim of unaltered cytoplasm. The nucleus, too, becomes reduced, but not in proportion to the cytosome. In a testis in which the interstitial tissue is degenerating all stages may occur, as illustrated in figures 12, 15, and 17. The nucleus of the degenerating cell is at first hyperchromatic, and as the cell shrinks it becomes pycnotic, embedded in a small amount of heavily staining cytoplasm. Such a cell resembles in many respects the dark granulosa lutein cell described in the human corpus luteum of pregnancy (Gillman and Stein, 1941).

Once initiated, degeneration proceeds at a great pace. In some testes entire masses of cells liquefy and are replaced by loose connective tissue, giving the impression that the tissue is badly fixed (fig. 17); but in such instances adjacent tissues show no evidence of autolysis. At the end of the acute degenerative phase only small clusters of cells remain. A similar type of massive liquefaction of cells is regularly seen in the deepest part of the wall of the lutein cyst (adjacent to the lining of the cavity) in the corpus luteum of early pregnancy (Gillman and Stein, *op. cit.*). There is little doubt that the fetal interstitial cells undergo a definite cycle of growth, development, decline, and degeneration. This phenomenon is too consistent to be abnormal, or peculiar to some gonads.

Unless a complete series of testes is available, from the beginning of differentiation until birth, an erroneous impression of the history of the interstitial cell may be obtained. If there are important gaps in the series between 54 mm. and 140 mm., it may appear that the interstitial cells remain small throughout development, only to enlarge immediately before puberty. It is remarkable how few texts refer to this astonishing cycle. Felix (1912) in his lengthy chapter on the development of the sex glands in Keibel and Mall's *Human Embryology* makes no reference to the interstitial cells except to say (vol. 2, p. 895):

> In embryos of 45 mm. greatest length the genitaloid cells are transformed into large pale cells (interstitial cells) which resemble genital cells in shape and size, but their nuclei contain only little or even no chromatin. These cells gradually disappear after the fifth month, and in fetuses of the seventh month they are still present here and there beside the vessels in the spaces between the testis tubules. After birth an abundant connective tissue develops between the tubules, and, as a result, the number of interstitial cells diminishes still further; after puberty they again appear to occur in increased numbers.

Simkins (1928), who studied the early development of the ovary and testis, was more concerned with the origin of the sex cells. He reiterated Felix' views and makes only a casual reference to the interstitial cell, to the effect that it is derived from the genitaloid cell, which is also incorporated into the seminiferous tubule. Stieve (1930), however, was deeply impressed by the great enlargement of the interstitial cell in the fetus.

In the testis, differentiation and growth of the interstitial cells occur during a phase of development when the ovary, as will be shown, increases only in size, with little evidence of morphological differentiation. When atrophy of the interstitial cells of the testis is under way, the ovary for the first time shows evidence of more rapid differentiation, with the appearance of fetal stroma in the deepest part of the medulla, and the formation of the first primordial follicles. Such a sequence of events in the ovary and testis can scarcely be coincidence; indeed, it must reflect some fundamental physiological process in sexual development. It is during this period that some of the well known anomalies are determined.

The salient features which emerge with respect to the interstitial cells are, first, that they arise from the mesenchymal part of the genital primordium, with perhaps a small contribution from the cells of the

tunic and the mesenchyme of the mesonephros; second, that they begin to differentiate as interstitial cells when the fetus measures about 30 mm. C.-R.L. Differentiation involves more and more cells, until at 54 mm. they constitute a large proportion of the gonad. Maximum development is attained between 130 and 160 mm., after which they suddenly decline until, at 286 mm., only a few small islands remain. They first appear in the most distal part of the intertubular space, beneath the tunic, and spread toward the rete region. The cells atrophy in the same order in which they appear.

## Seminiferous Tubules

The seminiferous cords are the most important single criterion for identifying the newly differentiated male sex gland. Sex cords are of course formed very early by the coelomic epithelium, and are long continuous with the cellular layer. These cords are numerous, but rather ill defined at first, separated by thin septa of mesenchyme. The cells are small and closely crowded together, the free ends of the cords resembling a syncytium, with the nuclei clustered in the center, around a clear zone. This arrangement foreshadows the formation of the rete complex. Although the sex cords in the ovary are less organized, such a stage nevertheless occurs.

As differentiation continues, the male sex cords become more compact and the cytoplasm is more in evidence. In a transverse section, the cell arrangement clearly sets off the seminiferous cords from the mesenchymal bed. The testis is now formed in miniature, but the cellular layer (representing the primitive tunica albuginea) is still in continuity with the seminiferous cords, which now converge toward the rete region. The newly formed testis grows rapidly. In a 27-mm. embryo it appears, in longitudinal section, as a much elongated body attached by a narrow mesorchium to the mesonephros (fig. 8). Four distinct zones may be identified: first, the outer cellular layer, covered by an irregular epithelium; second, the zone of sex cords, which often form wide arcades by anastomosis of their peripheral extremities; third, the zone of straight tubules, where the mesenchyme tissue is looser than elsewhere; finally, the rete blastema, forming the inmost zone. This delimitation into zones is significant, since it is also seen in the ovary, where the regions are comparable in many respects. Strictly speaking, in the young testis there is a cortex consisting of the first two zones, and a medulla comprising the straight tubules and the rete.

As the interstitial cells become more numerous, the seminiferous cords are more widely separated. This accentuates the anastomotic loops of the cords, while exaggerating the narrowness of the straight tubules. The seminiferous tubules remain widely separated in older fetuses. Thus in figure 10, from a fetus of 54 mm., the loops of testicular cords are well demonstrated, and the intervening beds of interstitial cells are conspicuous. The straight tubules are seen communicating with the rete. As a rule two or three seminiferous cords communicate with a single straight tubule, but sometimes as many as five may join. Growth of the tubule is more active toward the distal extremity, and it is here that coiling first begins, whereas the segment nearest the rete remains thin and straight (fig. 11). In this specimen tubules seem more numerous than in previous ones, but actually this appearance is due largely to coiling.

Throughout the growth period of the interstitial cells, development of the tubules is relatively slow, and the pattern simple. It appears that hypertrophy of the interstitial tissue, directly or indirectly, inhibits the growth of the cords. In figure 11, the testis has greatly increased in size, as compared with that in a much younger fetus (fig. 10), but the tubules are for the most part relatively straight. Not until the interstitial tissue is near its peak do some tubules first acquire a lumen; and only after great reduction of the interstitial cells does lumen formation proceed actively. In a fetus of 187 mm. C.-R.L. (fig. 14) the interstitial cells have passed their maximum and are beginning to degenerate; the tubules are now more coiled, especially adjacent to the tunic. Most of these tubules now have a lumen. The tubules then increase rapidly in complexity and encroach on the space previously occupied by the interstitial tissue. In a fetus of 286 mm. all are patent. In the fully formed testis the tubules are extremely tortuous, but this complexity is achieved only toward the end of gestation. In the testis of a newborn infant they are closely crowded.

It has been stated by various workers (Oslund, 1924, and others) that the interstitial tissue is reduced by the rapidly developing tubules, which are said to induce the atrophy by compression. This view is untenable, since even after marked atrophy of the

interstitium the tubules are still relatively small (fig. 16). In point of fact, there is a distinct lag between the first decline of the interstitial cells and the spurt in the growth of the tubules.

The constituents of the seminiferous tubule have two origins: from the coelomic epithelium and from the primitive sex cells, found within the mesenchymal primordia of the genital ridge. When the sex cords first appear as small buds growing into the compact mesenchyme, the cells are small, with moderately chromatic nuclei and scanty cytoplasm. As the cords enlarge, they become intimately associated with the primitive sex cells, which now diminish in size and are incorporated into the cords. Thereafter they cannot be readily distinguished from the other cells of the cord (fig. 6). The earliest parts of the cords contain but few sex cells, but the parts formed later contain many. After the primitive cords have become seminiferous cords, those sex cells not incorporated degenerate and disappear.

In embryos of 15 to 16 mm. the cells of the cord are compactly arranged, their long axes transverse to the longitudinal axis of the tubule. In outline they may be cone-shaped, pear-shaped, or cylindrical. The cytoplasm increases and the cell membrane becomes more visible. The central end of the tubule is for a time more highly differentiated. Much later, when the tubule is detached from the cellular layer (primitive tunica albuginea), two types of cells may be recognized. The commoner form is elongated, with a small oval nucleus; the other is a large spheroidal cell with clear cytoplasm and a large round or oval, vesicular nucleus. Cells of the latter type are more numerous in the distal half of the tubule, diminishing in number toward the rete region, where only an occasional one occurs. These cells have all the features of spermatogonia. They are never abundant in early stages, but later become more numerous, and may reach a considerable size. They usually lie next to the basement membrane of the tubule (fig. 13), and are a prominent feature in older specimens (fig. 17).

Cells of the more numerous nongerminal types line the walls of the rete tubule and straight tubule. The fact that the rete canals and straight tubules develop from the first-formed segments of the sex cords is significant, for these segments arise at a time when the primitive sex cells are few. Conversely, the last-formed segments, which develop when the primitive sex cells are more numerous, contain an abundance of germ cells. It is most unlikely that these primitive sex cells are incorporated in the seminiferous tubules only to degenerate (being replaced allegedly by cells from the coelomic epithelium, in common with the Sertoli cells and the lining epithelium of the rete and straight tubules). In the testis it is, in fact, difficult to follow their fate with certainty, because of their small size after incorporation. In the ovary, however, they unmistakably persist as oögonia.

Stieve (1930) maintains that the coelomic epithelium gives rise to both the sex cells and the Sertoli cells. Simkins (1928) states positively that the coelomic epithelium forms indifferent cells and genitaloid cells. The indifferent cells become Sertoli cells and permanent sex cells; some of the genitaloid cells are incorporated in the sex cords and later degenerate. The remainder of the genitaloid cells outside the tubule persist to form Leydig cells. Felix, however, is prepared to admit that primitive sex cells migrate from some extragenital source to enter the genital primordium, but believes that these cells there degenerate, to be replaced by the permanent sex cells, derived from the coelomic epithelium. Simkins denies the existence of the primitive sex cells. This writer may have been unfortunate in the selection of his material, for these cells have been found in all early human embryos which the author has examined (figs. 1–6). Stieve is at a loss to account for the large cells seen below the coelomic epithelium of the genital ridge. The evidence from comparative studies reveals that the primitive sex cells in a large variety of vertebrates migrate into the primordium of the gonad from some extragenital source, and are incorporated secondarily into the sex cords.

The coelomic epithelium therefore gives rise to the lining epithelium of the rete testis, the tubuli recti, and the seminiferous tubules; in the latter the lining cells are the Sertoli cells. The sex cells arise from some extragenital source, and their immediate precursors are the primitive sex cells, so numerous in the genital primordia of young embryos (figs. 1–6). The interstitial cells are derived largely from the compact layer of mesenchyme beneath the thickened coelomic epithelium, probably supplemented later by cells from the mesenchyme of the mesonephros.

## Rete Testis

The youngest embryo in which the rete appears as a definite structure measures 10 mm. in length, but this tubular complex is foreshadowed in younger embryos. Soon after the free ends of the more cranial sex cords have penetrated the compact layer of mesenchyme, they bend sharply and grow in a caudal direction. Eventually almost all the sex cords are thus involved, and converge toward the mediastinum.

Incipient differentiation of the free extremities into rete cords is indicated when the cells become more closely compacted and the nuclei more hyperchromatic than elsewhere in the cord. Sometimes the cells are aggregated as in rouleau formation. When the sex cords proper differentiate into seminiferous tubules, the rete complex is the more conspicuous because of its denser structure.

The rete cords maintain a central position and, when the testis elongates, occupy the medullary region from the mesorchium almost to the tip of the section (fig. 8). The small, narrow cords are enveloped in loose mesenchyme. Cell boundaries are so indistinct that under high magnification the clusters of nuclei appear to lie in a common mass of cytoplasm. Although the rete cords are especially accentuated in the testis, they show but little transformation until the fetus has reached 40 mm. C.-R.L. They do become more complex by anastomosing freely, but lumen formation begins only when the fetus is about 100 mm. in length.

In early development, the sex cords elongate chiefly by accretion at the periphery, causing the gonad to bulge more and more into the peritoneal cavity. Growth in the opposite direction is not easily possible because of the position of the mesonephros. Even before sexual differentiation occurs, the rete cords are not clearly separated from the glomerular capsules; and as soon as the testis can be recognized histologically, some cords are in contact with those glomeruli which are to become part of the duct system. In some embryos of 30 to 40 mm. C.-R.L. the rete cords appear to be in continuity (via glomerulus and tubule) with the mesonephric duct. When the narrow lumen of the rete can be identified, in fetuses over 100 mm. in length, there is almost invariably a close union. In none of the many specimens examined did the rete cords join any other part of the mesonephric tubule. The vasa efferentia, therefore, include the whole mesonephric unit—glomerulus, tubule proper, and collecting tubule.

Even before the rete cord joins the glomerulus, the glomerular tuft is reduced in size and the capsular space increases, so that the small tuft is prominently outlined. It later disappears completely. On rare occasions a small outpouching of the glomerular capsule occurs at the junction with the rete cord, so that a short segment of the cord consists of cells derived from the capsule.

Despite the fact that the rete cords are derived from the coelomic epithelium, they rarely contain germ cells. This is accounted for by the fact that they develop rapidly and as a rule before the primitive sex cells have become incorporated into the sex cord. When a germ cell happens to be included in the wall of the rete cord, it contrasts strikingly with the small cells of the latter. It should be reiterated that the interstitial cells in general stop sharply in the region where the straight tubules merge with the rete tubules. When the interstitial cells reach their maximum development, the rete region presents a remarkable appearance, with the large interstitial cells on one side and the small, star-shaped mesenchymal cells on the other. Only rarely is a large interstitial cell closely associated with the anastomosing rete cords. Such cells tend to be encapsulated by the mesenchyme.

There was no evidence from the large series of human embryos examined that the rete cords were derived from the mesonephric tubules, or from the mesenchyme of the mesonephros; neither was there any indication that the cranial region of the genital ridge specialized to form cords which connected secondarily with the seminiferous cords. The more cranial sex cords differentiate first, and it is true that when they have penetrated the mesenchyme they bend caudally to form later a portion of the rete complex; but it is also true that the more caudal cords bend cranially to contribute to the rete.

The rete cord, straight tubule, and seminiferous tubule are derived from one continuous structure, the sex cord, parts of which arise at different periods in time. The rete region develops first, and the seminiferous tubule proper is the last to be formed.

## DEVELOPMENT OF THE OVARY

In the development of the ovary such notable modifications of structure occur from time to time that it is impossible to avoid subdividing the course of events into definite stages. These are: (1) indifferent stage, (2) stage of sexual differentiation and growth, (3) stage of formation of fetal stroma and primary follicles, (4) stage of mature stroma formation. The last is postnatal. Each of these stages merges imperceptibly into the next, but once established the morphological features are distinctive, and represent important landmarks in ovarian development.

### Indifferent Stage

The indifferent gonad presents minor divergences in morphology. These may represent sexual differences or may be merely individual variations, for such variations exist in the embryo as in the adult.

It is impossible with available techniques and methods to interpret with certainty all such differences. This is the inevitable handicap encountered when tissues must be removed for histological study at a given stage in time. The complete story can be reconstructed only by the use of a large series of specimens of different ages, and even under ideal conditions such a reconstruction of events may still be open to criticism. The general appearance of the gonad in the indifferent stage has already been fully described above; it has features common to the two sexes, although in structure it is closer to a testis than to an ovary.

### Stage of Sexual Differentiation and Growth

The metamorphosis of the indifferent gonad into an ovary is not as spectacular as in the case of the testis, nor is it as easily recognizable. In the testis the sudden accentuation of the sex cords and the rapid disappearance of the primitive sex cells from the outer cellular layer are notable features. The differentiated testis at least bears some resemblance to the fully formed gonad. The recently formed ovary, however, in no wise resembles the fully developed organ and can be recognized only by negative criteria, namely, the absence of prominent sex and rete cords and of any modification in the outer cellular layer. Perhaps the persistence of a large number of primitive sex cells in the outer cellular layer may be considered characteristic. The young ovary is thus identified chiefly by the fact that it is not a testis.

In an embryo 17 mm. long the cells of the sex cords near the outer cellular layer have increased in size and the intervening strands of mesenchyme are inconspicuous (fig. 20, pl. 4). The coelomic epithelium is not sharply demarcated from the underlying cellular layer, in which are found large primitive sex cells and smaller, more numerous cells derived from the epithelium. The latter will ultimately encapsulate the primitive sex cells, and are named pregranulosa cells. Their nuclei are more vesicular than those of the coelomic epithelium, but transitional forms exist (fig. 20). Throughout the growth stage, which lasts until the embryo has achieved a size of 100 to 130 mm. C.-R.L., the coelomic epithelium continues to add new pregranulosa cells to the enlarging ovary. The outer cellular layer, moreover, continues to grow in thickness and is not sharply delimited from the surface epithelium.

In the superficial zone of the ovary of the 17-mm. embryo (fig. 20) four types of cells are present, namely, (1) the primitive sex cells, (2) the pregranulosa cells, (3) cells of the coelomic epithelium, (4) spindle-shaped mesenchymal cells. The primitive sex cells have increased in size as compared with those seen in the indifferent gonad (fig. 6). The cell boundaries are more sharply defined and the nucleus is relatively smaller. The cytoplasm is slightly granular, and lying near one pole of the nucleus, in the position of the Golgi apparatus, is a mass of small eosinophilic granules. The nucleus is vesicular, but the prominent nucleolus, so typical of the primitive sex cell of the indifferent gonad, is no longer present. From careful examination of primitive sex cells in the indifferent gonad and in the young ovary there is nothing to indicate that they arise from the somatic cells of the coelomic epithelium. It is obvious that the large cells of figures 6 and 20 are virtually identical. In figure 20, no cells transitional between the coelomic

epithelium and the primitive sex cells could be found, but forms intermediate between surface cells and pregranulosa cells are common. The latter as a rule have more vesicular nuclei, are more widely separated, and have better-defined cell boundaries than the parent cells. In the more superficial regions the pregranulosa cells are diffusely scattered, but in the deeper part of the cellular layer they occur in clusters, usually associated with several primitive sex cells. These clumps correspond in position to the outer ends of the seminiferous tubules, near the primitive tunica albuginea. In this region spindle-shaped mesenchymal cells can be seen skirting the cell clusters (fig. 20).

Deeper within the ovary, in a position intermediate between the cortex and medulla as described by some investigators, and corresponding to the zone of seminiferous tubules in the testis, the sex cords are ensheathed by spindle-shaped mesenchymal cells (fig. 21, pl. 4). These cords are more massive than those of a much older male fetus (fig. 9), a fact which explains why the cellular layer is much thicker than the corresponding layer in the testis. As already mentioned, there is a general increase in size, both of the sex cells and of those derived from the coelomic epithelium, extending as far as the level where sex cords and rete cords merge. Cells derived from the coelomic epithelium, however, are smaller than the sex cells. Thus, the large sex cell in figure 21 is at least twice the size of its neighbors. The cell bodies of the true cord cells are much more sharply defined than at any previous stage; and the thickening of the sex cords has so reduced the mesenchymal septa that the configuration of the cords is obscured. The cells of the rete complex, however, remain small and compact, so that the rete cords contrast strikingly with the rest of the ovary; but continuity with the sex cords can always be observed in well fixed ovaries.

Although the mesenchymal septa are attenuated in comparison with younger stages, in the rete region this tissue is abundant, diminishing progressively toward the surface of the ovary. In some sections (figs. 20, 21) it might appear that the sex cord is separated from the more superficial cellular zone by a distinct layer of mesenchymal tissue. Such a layer has been called the primary tunica albuginea, and is said to correspond to the tunica albuginea of the testis. Actually, such separation is more apparent than real, since much depends upon the plane of section. The cellular layer is continuous with the sex cords by relatively narrow stalks, not always seen in single section. Thus, the contrast between the mass of cells in the outer region and the rete cords may be markedly accentuated. Moreover, spindle-shaped mesenchymal cells later penetrate the cellular layer to form the tunica albuginea of the ovary.

It will be remembered that the tunica albuginea of the testis is differentiated from the cellular layer (corresponding to the cortex in the female; compare figs. 9 and 20). The term primary tunica albuginea is unjustified, for it gives an erroneous impression of the origin of the tunica albuginea in the male. The so-called primary tunic in the ovary and the tunica albuginea in the testis cannot be directly homologized. The connective-tissue septa which separate the sex cords of the indifferent gonad, and which are accentuated in the male when the seminiferous tubules appear, are still present in the ovary as we have seen, but are not easily recognized, save at wide intervals. In later stages, the reticulum becomes more prominent, with the growth of the blood vessels (fig. 27, pl. 5).

The young ovary therefore consists of a covering layer of coelomic epithelium which merges imperceptibly with the underlying cellular layer. The sex cords are much thicker because of a general enlargement of the constituent cells, but remain in continuity with the rete cords. The primitive sex cells, a prominent feature of previous stages, are still easily seen in the massive sex cords (fig. 20). The innermost segment of the sex cord, where it joins the rete cord, represents the medullary cord of later stages. It may differentiate into small follicles which may or may not disappear before birth.

Once the indifferent gonad has become an ovary, a prolonged phase of growth follows, attended by multiplication and enlargement of the pregranulosa cells, and proliferation and reduction in size of the primitive sex cells. This growth phase is one of the most remarkable features in the development of the ovary. It commences soon after differentiation begins, and continues until the fetus has attained a crown-rump measurement of over 100 mm. There is scarcely any modification in structure throughout this long period. In the comparable period the testicular cords grow very slowly, while the interstitial cells rapidly differentiate and enlarge to form the enormous bed which

has been described, a development without its counterpart in the ovary.

Throughout this period the ovary steadily increases in size. At first the gland is bean-shaped in cross section (fig. 22, pl. 4) and is much elongated in longitudinal section (fig. 18, pl. 3). But for the absence of the prominent sex cords, it resembles a testis of about the same age (compare figs. 8 and 18). Cords are in fact present, but are not easily identified under low magnification (fig. 23, pl. 4); so the outer cellular layer seems to have increased in thickness at the expense of the zone of sex cords. Actually, proliferation of the sex cells occurs equally in the depth of the ovary and in the more superficial parts. In figure 23, mitotic figures are found even in the area near the rete cords, but these are in the sex cells almost exclusively. The increase of sex cells, therefore, contributes to the enlargement of the sex cords and the attenuation of the mesenchyme, so that the whole ovary, external to the small region close to the rete cords, appears merely as a thickened cellular layer. The rete cords are ill defined and undergo no change during this period. They may become relatively smaller as compared with those of the testis (figs. 18, 23; fig. 8).

In addition to steady growth, the other important event in the ovary is the reduction in size of the primitive sex cells and the slight enlargement of the pregranulosa cells, giving the cellular bed a more uniform texture. This can be recognized by comparing the ovary of a fetus 31.5 mm. long (fig. 23) with that of one measuring 109 mm. (fig. 25, pl. 4), but it is seen more clearly in high-power views of the ovaries of 17-mm. and 109-mm. fetuses (figs. 20, pl. 4; 26, pl. 5). In the older specimen the large nuclei of the sex cells with their complicated chromatic networks are noteworthy (fig. 26). The cordlike arrangement of the cells seen in figure 20, from a 17-mm. fetus, is no longer to be seen. In both specimens the surface epithelium is irregular and cells are being steadily added to the underlying layer; the prefollicular cells are dominant. In general these two ovaries have more or less the same structure, despite the fact that one fetus was only one-seventh as long as the other.

Capillaries and connective-tissue cells are scanty except in the rete zone. Despite the fact that in fetuses over 100 mm. in length the gonad contrasts obviously with a testis, there is still no structural resemblance to the ovary of older fetuses. It can be realized, therefore, why this stage in the development of the ovary has been named the stage of sexual differentiation and of growth. It is significantly different from the preceding indifferent stage, as well as from the succeeding period of formation of fetal stroma and primary follicles (compare fig. 6 with fig. 25, and fig. 25 with fig. 28).

### Stage of Formation of Fetal Stroma and Primary Follicles

It is during this stage that the female gonad first begins to resemble an ovary. This happens when the fetal stroma invades the medulla and spreads into the central region. Encapsulation of the oögonia by the follicular cells precedes the invasion of the stroma, primary follicles appear first in the medullary region, and the outermost layer of the cortex is modified last of all. The fetal stroma is seen first in the medulla of fetuses measuring 140 to 150 mm., and may extend throughout the ovary in fetuses of 280 to 300 mm., by which time the first follicles with cavities make their appearance in the medullary region.

The earliest evidence of the fetal stroma takes the form of a condensation of mesenchymal cells in concentric layers around the blood vessels and rete tubules, supported by a reticulum seen even in haematoxylin and eosin preparations. This reaction is encountered in fetuses from 90 to 100 mm. in length, although the ovary proper is not yet affected (figs. 25, 26). Later the vessels of the mesovarium and medulla are more prominent, and even in the cortex the capillaries are more easily identified than previously. At this stage some of the oögonia in the deeper part of the cortex begin to degenerate. The cells enlarge, the cytoplasm shows vacuoles, and the hyperchromatic, irregularly shaped nucleus is pushed against the cell wall. Degeneration of oögonia is much more obvious in later stages (fig. 27).

In fetuses of 140 to 150 mm. the condensation of mesenchyme seen first in the rete region extends into the ovary. Immediately before this event the oögonia in the medulla enlarge and are encapsulated by prefollicle cells, and thus the first primary follicles appear. Only after the oögonia are encapsulated does

the fetal stroma invade the medulla and the contiguous parts of the cortex. The follicle cells become so flattened in the process of encapsulation that they may resemble stromal or even endothelial cells. The fact that oögonia acquire follicular capsules before the stroma invades the ovary is crucial evidence that these cells are not of stromal origin.

In this stage of differentiation two processes are involved: first, the enlargement of oögonia and their encapsulation; and second, the progressive extension of the stroma into the ovary. As the stroma advances, unencapsulated oögonia enlarge, become vacuolated, and ultimately degenerate. This phenomenon is encountered in the cortex as well as in the medulla.

In a 190-mm. fetus the stroma has already appeared in the deepest part of the medulla (fig. 27). Here the enlarged oögonia are surrounded by flat granulosa cells which on superficial examination appear to be derived from the fetal stroma ovarii. This in fact is not the case, as all granulosa cells are large at first and only later assume a flat shape. Coincidently with the emergence of the fetal stroma, the capillaries of the cortex, hitherto quite inconspicuous, are greatly accentuated by a connective-tissue investment. Septa of connective tissue are present much earlier, but can be seen only in favorable sections. They represent that part of the mesenchyme which enveloped the sex cords in the indifferent stage. These septa now extend throughout the cortex and spread out below the investing epithelium to form the tunica albuginea. Sometimes the surface epithelium forms a shallow fold, which extends into the cortex. In such regions the tunic becomes very thick and is usually continuous with a thick septum. The septa divide the ovary into a number of thick strands or cords.

The cell masses thus partitioned by septa resemble at this stage the "tubes of Pflüger." These cords are evident in the more superficial parts of the ovary, but toward the hilum become more attenuated; in the medulla they are very slender. There is always continuity between the cords of the medulla and those in the cortex (fig. 27), although in oblique sections the cortex appears to be separated from the medulla by a connective-tissue layer, the "primary tunica albuginea" of some writers. In figure 27 some cell masses within the cortex are apparently thus set off, but in other areas the continuity between the two regions is clearly evident. There can be little doubt that the "primary tunica albuginea," if it exists at all, is very imperfect. It is never well defined in young ovaries.

Figure 27 demonstrates yet another important phenomenon, the massive degeneration of the oögonia just in advance of the invading stroma. This degenerative process varies in degree from ovary to ovary and is correlated with the number of follicle cells. When the germ cells are disproportionately numerous and the follicle cells are insufficient to encapsulate an oögonium or group of oögonia, then degeneration of the sex cells invariably occurs. The fetal stroma seems to exert a lethal action on naked eggs, or at any rate is incapable of providing a proper environment.

The fetal stroma condenses most rapidly in those areas where it first appears, and proliferates actively as it invades the cortex. Figures 28 and 29, plate 5, are, respectively, sections through the cortex and through the junctional zone between cortex and medulla of a 221-mm. fetus. In figure 29 the stroma is abundant as compared with that in a younger fetus (fig. 27). The primordial follicles are larger and the follicle cells still form but one layer, but they are now less flat than when the stroma first invaded the ovary. At *mc* is the remains of what would be regarded as a medullary cord, but close scrutiny reveals the presence of some oögonia and numerous cells of the follicular type. Many oögonia are degenerating, and the follicle or granulosa cells are concentrated around those surviving. Some oögonia may persist to develop later into medullary follicles. In this connection it may be mentioned that rete cords may also contain an occasional oögonium, but as a rule the oögonia die and the surrounding cells persist as the epithelium of the rete tubule. The apparent disappearance of the medullary cords at this stage of development is due to two factors: first, the great increase in the fetal stroma, and, second, the enlargement of the oögonia and the rearrangement of adjacent cells to form the granulosa.

The medullary cords therefore are not the remains of the seminiferous tubules, but represent rather the vestiges of the primitive sex cords, specifically the segments corresponding in the male to the straight tubules. Only in a topographical sense, however, is this homology between medullary cord and straight tubule possible, not in any other way. The first parts of the male sex cords, from which rete tubules and straight tubules are developed, contain few germ cells

and are more highly organized. They are ensheathed very early by the mesenchyme. In the female also the first parts are poorly endowed with sex cells, and they too form definite cords with a sheath of mesenchyme. When the sex cells in the ovarian cords enlarge at the time the stroma appears, cord cells (derived from the coelomic epithelium) attempt to encapsulate the oögonia. The peculiar structure of the cord to a certain extent restricts this process, so that the oögonia die and the cords are largely disrupted; or if there are few oögonia the prefollicle cells may persist as a medullary cord. Occasionally the more viable cells persist for a while as small dark-staining masses. Such clusters of cells may also be found later in the superficial layers of the cortex (fig. 30, pl. 5; figs. 31, 32, pl. 6), where they form cords, rudimentary tubules, or structures resembling the anovular follicles described by League and Hartman (1925) in the opossum, and by others in a variety of animals. These cords in the cortex of the ovary will be examined in greater detail in due course.

The importance of this stage in development, as revealed in figures 28 and 29, is that the original structure of the ovary is once again brought into relief by the thickening of the pre-existing septa. The cords are extremely thick in the cortical region and narrow markedly in the medulla.

The long latent period before the female gonad becomes a typical ovary, with stroma and follicles, renders it particularly subject to abnormalities which cannot easily occur in the male, since the testis differentiates early. A noteworthy feature of ovarian development is the existence of a gradient of differentiation. In figure 31 attention was drawn to the large primordial follicles which appear first toward the hilum, whereas elsewhere the oögonia are smaller and the prefollicle cells are distributed irregularly. Follicles are always absent from the cortex (fig. 28). Moreover, the primary follicles become multilayered in the medulla long before they do so in the cortex. Toward the end of gestation the medullary follicles develop fluid and undergo atresia, but such a phenomenon is rare in the cortex. Very frequently the granulosa cells appear to be insufficient to provide a capsule for all oögonia, and groups of two, three, or more may be encapsulated collectively. Where the stroma is invading the ovary, polyovular primary follicles are extremely common. As a rule they die later, but some may persist as polyovular Graafian follicles.

In fetuses measuring 260 to 280 mm. the stroma has almost reached the tunica albuginea. In some cases the pregranulosa cells display an extraordinary reaction, with enlargement of the cell and marked hyperchromasia of the nucleus (fig. 30). This condition contrasts with the flat form of the granulosa cell in a younger fetus (fig. 27). Sometimes the reaction is intense, and the cell masses then resemble tumors of the granulosa cell type (figs. 31–34, pl. 6). The nature of this reaction was at first puzzling, but after many ovaries were examined and the process was seen in all degrees (from the mild type shown in figure 30 to the extreme form illustrated in figure 34), its meaning became evident.

Attention has been drawn to the apparently lethal effect of the fetal stroma on naked oögonia. When the septa become prominent in fetuses of 160 to 220 mm., the stroma is already present in the hilum and part of the medullary region. At this time pregranulosa cells commence to surround the oögonia. If they are insufficient to provide capsules for all oögonia and the source of supply is likewise inadequate, then many oögonia degenerate, sometimes on a grand scale. In figure 27, for example, at least every second oögonium is vacuolated and the nucleus pyknotic. In some ovaries in which degeneration is prevalent, the surface epithelium is stimulated to renewed activity. This may affect part or all of the epithelium, and here and there groups of cells may be seen undergoing mitosis. In figure 32 the surface epithelium has become active in the bottom of folds. The resulting cells, in the form of cords or alveoli, invade areas crowded with oögonia. In this specimen, too, stroma is absent from the cortex, although the septa are prominent and the tunica albuginea is relatively thick.

At about the time the stroma has appeared throughout the ovary, the primary follicles in the medulla begin to enlarge, chiefly by the formation of additional layers of granulosa cells. Once multilayered follicles are formed, production of fluid is imminent. The appearance of fluid and of true Graafian follicles suggests that the ovary has become responsive to gonadotrophic hormones.

Gonadotrophic hormones may thus produce their effects at a time when not all the oögonia are encapsulated or before newly formed pregranulosa cells

have established contact with the oögonia. This may result in masses of cells taking the form of cords, tubules, or alveoli. When such pregranulosa cells are few the reaction is mild (fig. 31), but when they are numerous it is very intense, as in figures 33 and 34.

These curious formations are found at the periphery of the ovary, near the hilum, and in deep folds of the coelomic epithelium. Their activity supports the view that the granulosa cells are derived from the coelomic epithelium and not from the stroma. Clumps of cells with hyperchromatic nuclei persist until birth and even for several months after. They are similar to the "Eiballen" of Meyer. Such cells may give rise to anovular follicles, cysts, and probably tumors, certain types of which (figs. 33, 34) they much resemble.

The cells of the coelomic epithelium continue to furnish pregranulosa cells until the fetus is about 280 to 300 mm. in length. The amount of proliferation varies even in different parts of the same ovary. When there is slight mortality among the oögonia, the surface epithelium proliferates to some extent as the fetal stroma invades the ovary; only in cases of mass degeneration of oögonia is the proliferation excessive. Even in such cases the epithelium in one region may react normally, whereas in another there is only a localized reaction. Many ovaries show a response of this character only in the ring of tissue surrounding the attachment of the mesovarium, where small, fibrous excrescences apparently represent the remains of an excessive proliferation.

When proliferation has been excessive over the whole ovary, the fibrous reaction may also be intense. The tunica albuginea, normally a thin layer, may become a dense coat, as shown in figure 33 and especially in figure 34. This type of fibrous tunic is frequently seen in fetal and postnatal ovaries. In extreme cases it may persist beyond puberty and offer a serious impediment to ovulation. Such a congenital abnormality may be a cause of fibrocystic disease.

The fetal stroma thus appears in the hilum of the ovary when the fetus has attained 150 to 180 C.-R.L., and permeates the whole ovary at 280 to 300 mm. It is significantly different from the adult type, which appears some time after birth; the fetal stroma cells are spindle-shaped, with relatively large spherical or oval nuclei (figs. 28–30), whereas in the fully differentiated stroma the cells are extremely flat and the nuclei elongate. As soon as the stroma has spread almost throughout the ovary, the primary follicles acquire a capsule of stroma cells. The granulosa cells now proliferate to form a multilayered *stratum granulosum,* and produce fluid, which is often retained within the cells for some time. The follicles assume a characteristic appearance rarely seen in postnatal ovaries. Eventually the fluid leaves the cells to accumulate in the follicular cavity.

The capsular tissue differentiates into theca cells, which may form several layers around the follicle. In atretic follicles, the theca cells may hypertrophy to such an extent that they are almost indistinguishable from the large interstitial cells of the male, at 100 to 140 mm. This type of hypertrophic theca cell occurs in atretic follicles in the human ovary during pregnancy (Gillman and Stein, 1941).

Atresia of the fetal follicle is usually attended by less fibrosis than in the adult ovary. Toward the end of gestation some follicles may become cystic, and the thecal hyperplasia which invariably follows is like that of cystic follicles associated with hydatid mole, or chorioepithelioma. It is not possible at present to account for this intense reaction of the theca cells.

The youngest specimen in which definitive follicles were found measured 254 mm. in length. After this period more and more appear. Some are small, others may become cystic.

In the adult human ovary the growth of follicles and the conversion of the ruptured follicle into a mature corpus luteum is generally regarded as a visible expression of endocrine action. Similarly, the encapsulation of oögonia by pregranulosa cells and the evolution of the Graafian follicle may also indicate the presence of circulating gonadotrophic hormone in the fetus. The intense thecal reaction in atretic follicles also suggests hormone activity, since degeneration of follicles in hypophysectomized animals occurs without such hyperplasia. This third stage of ovarian development is initiated at the time when the testis begins to lose its interstitial cell bed. Atrophy of the interstitial cells is almost complete at 280 mm., after which the tubules gradually become more tortuous, and encroach on the space previously occupied by interstitial tissue. It is significant that follicle formation also first appears when the fetus is about 280 mm. in length. There is nothing in the ovary corresponding to the hyperplasia of interstitial cells in the testis. Actually, the second stage in the ovary can almost be

regarded as a stage of inhibition of the differentiative processes, since growth alone is characteristic of this long period, during which such spectacular changes occur in the testis. The origin of the gonadotrophic hormones in the fetus will be discussed later.

The developmental stages outlined above have an indisputable morphological basis, although, as will be recognized, the transitions are not abrupt. This classification is more than a convenience, since it emphasizes outstanding events in the development of the human ovary, and at the same time suggests the mediation of physiological processes which induce like modifications of structure in the adult organ. The sequence of events is so consistent that it obviously lends itself to experimental investigation.

## DISCUSSION

### The Germ Cells

The developmental origin of the germ cells has been the subject of much discussion and more speculation. Ever since Weismann (1883, 1904) enunciated his much disputed theory of the continuity of the germ plasm, investigation has centered around this lively question. The inevitable result has been a sharp cleavage of opinion, with a notable array of individuals taking opposite sides, while an equally numerous group prefers some form of middle course. Such division of opinion is mainly due to the limitations of the methods used. Two approaches are available, the morphological and the experimental. The morphological approach (i.e. the examination of presumably normal embryos, preserved at fixed intervals) has been the more popular, and the only method available to the older investigators; besides, the problem had to be understood and defined more precisely before it could be solved. Only in recent years have experimental procedures been sufficiently improved to be used reliably.

The study of fixed and sectioned material, especially if its purpose is to indicate the origin and destiny of single cells, is fraught with danger, as it is impossible to follow continuously the peregrinations of individual cells. The story must be pieced together from evidence furnished by different embryos, which may or may not be seriated correctly. This procedure also may not sufficiently take into account individual variation, which may be so marked as to constitute an anomaly. Even with an adequate series of embryos the difficulties by no means end, especially when the study involves the recognition of germ cells.

For the identification of cell types, even in adult organs, valid and generally accepted criteria are necessary, otherwise the personal factor interferes with interpretation. Many criteria have been proposed for identifying embryonic germ cells; these include size, the arrangement of the mitochondria and Golgi apparatus, the nature of the attraction sphere, and the structure of the nucleus. One or more of these has been used by various investigators. According to Hargitt (1925) size alone is no valid criterion, for all cells tend to round off before mitosis, even mesenchymal cells. He is skeptical of size alone as an important characteristic of germ cells, although obviously ova tend especially to be larger and better defined than somatic cells. Simkins (1928) also cautions that large blood cells may be mistaken for germ cells. The size of germ cells, however, has been emphasized by many investigators, especially in fish, amphibia, and birds (Beard, 1900; Woods, 1902; Richards and Thompson, 1921; Swift, 1914; Okkelberg, 1921; Witschi, 1914; Allen, 1904; Jarvis, 1908; Hann, 1927, and others). Even in mammals the germ cells are said to be relatively large (Vanneman, 1917; Felix, 1912). In man, size is unquestionably an important criterion, for nowhere else in the body have cells been found to compare in size with the enormous cells of figure 5, which cannot be confused with blood cells, nor with mesenchymal cells preliminary to mitosis.

The details and arrangement of the mitochondria are unsatisfactory criteria, despite the fact that Rubaschkin (1910, 1912) and Tschaschin (1910) found them of value. Others (Berenberg-Gossler, 1914, and more especially Swift, 1914) have been unable to recognize any differences between the mitochondria of somatic and of germ cells. Mitochondria vary greatly according to the physiological activity of the cell (Cowdry, 1918). The form of the Golgi apparatus also is extremely variable in different stages

of activity, as can be well observed in the granulosa lutein cell of the human corpus luteum (Gillman, 1939).

The large attraction sphere undoubtedly is a notable feature of germ cells, as Rubaschkin (1910), Berenberg-Gossler (1913), and Swift (1914) stressed. Its peculiar features require good fixation and special staining, however, conditions not always found in collections of embryos not specially prepared. In haematoxylin and eosin preparations this structure is usually not of much value.

The structure and size of the nucleus are valuable criteria, especially in later stages of development, as emphasized by Winiwarter and Sainmont (1909). Even in very young embryos the nucleus is large, in keeping with the size of the cell. Cell size may be useful in identifying early germ cells, but one may be certain only if the suspected cell can be followed continuously until it ultimately becomes a definitive germ cell.

Regarding the independence of germ cells and somatic cells, Hargitt (1925) rightly demands a satisfactory answer to three questions: "(1) Are primordial cells segregated at the beginning of ontogeny, i.e. in early cleavage stages? (2) If so, may these cells be certainly traced without a break to the definitive germ cells of the adult? (3) May any cells of the body, at any time, transform into germ cells?"

It may be possible to answer questions 1 and 3, but with the inadequate techniques available it is virtually impossible to answer question 2, and any attempt can be, at most, only a close approximation to the solution and nothing more.

Investigators who have expressed opinions concerning the origin and destiny of the germ cells fall into four groups, as presented by Heys (1931):

(1) Those who deny early segregation of germ cells and believe that germ cell formation is a matter of the differentiation of somatic cells. (2) Those who admit early segregation of germ cells, but conclude that such cells are not definitive but degenerate, to be replaced by proliferation of new cells from the germinal epithelium. (3) Those whose investigations have led to the conclusion that germ cells are segregated early and migrate to the site of the developing gonad. These persist as definitive germ cells but their numbers are increased periodically by a proliferation from the epithelium. (4) Those who believe that the definitive germ cells are set aside at an early stage in embryonic development, not to be replaced later by transformation of differentiated peritoneal cells. Their numbers are increased only by mitotic division.

In the Invertebrata and especially in the Vertebrata from fish to birds, the accumulated data thus far indicate that primordial germ cells do not originate in the coelomic epithelium, but migrate into the gonadal primordium from some extragenital source. In *Sphenodon* (Tribe and Brambell, 1932) and in the chick (Swift, 1914, and others) it seems almost certain that the primordial cells arise chiefly from a crescentic area of the blastoderm in front of the head of the embryo. They migrate to the genital primordium or are carried there by the circulation. In *Sphenodon,* apparently, active mitosis occurs within the genital ridge, during which the yolk spherules are absorbed and the cells enter on the prophase of the reduction division. In some cells characteristic yolk spherules can still be observed in synizesis. Brambell states that these are germ cells, although he wisely concludes that "it still leaves in doubt the question whether they give rise to definitive ova or spermatozoa."

Experimentally, important evidence supports the thesis of the extragenital origin of the germ cells in the chick. Reagan (1917) excised the crescent-shaped area containing the germ cells at an early stage, and the gonad then developed without germ cells. These observations were confirmed by Willier (1927), Willier and Rawles (1935), Benoit (1930), and Dantschakoff (1931).

In mammals the evidence for the extragenital origin of the primordial cells is not as positive as in other vertebrates. In reviewing the field Heys (1931) cautiously concludes, "There is still some evidence that Weismannian continuity may be a tenable hypothesis."

In this study of the development of the human gonad, the author was not preoccupied with the validity of Weismann's theory, but an explanation had to be found for the very large cells lying beneath the coelomic epithelium in the gonad-forming region. These cells were found there some time before the coelomic epithelium showed any evidence of proliferative activity. At first they were few, but coincidently with the formation of the sex cords they multiplied rapidly (fig. 5). Nevertheless the cells remained large and for some time preserved their original structure. No similar cells were to be seen in the coelomic epithelium, and there was no cogent evidence that

they entered the epithelium secondarily. There was clear evidence, however, that as the sex cords differentiated, the descendants of these cells mingled intimately with the cells of the cords (fig. 6), and even when the gonad formed a discrete mass, they were still most numerous in the region well below the surface (fig. 5). In the male all cells of the sex cords proper were extremely small, with no indication that the germinal epithelium produced other than a single type. Simkins (1928) and especially Stieve (1930) hold that all constituents of the sex cord in the human testis are derived exclusively from the coelomic epithelium, from which ultimately both Sertoli cells and germ cells arise. Stieve, however, recognized the large cells in the genital primordium, but regarded them as enlarged mesenchymal cells which later reverted to type and thus disappeared. Simkins saw these cells (genitaloid cells) only after the sex cords appeared, whereas Stieve portrayed them even before the genital rudiment was differentiated. Some, according to Simkins, were incorporated into the sex cord, where they later degenerated; others persisted as interstitial cells, and still others degenerated *in situ*. Stieve stated, moreover, that they disappeared early, and he derived the interstitial cells from mesenchyme. Thus Simkins and Stieve are at variance as to the origin and fate of the large cells in the genital rudiment, and as to the source of the interstitial cells as well.

The large cells in the primordium of the human gonad have been observed by many others (Kohno, 1925; Fischel, 1930; Fuss, 1912; Politzer, 1928; Felix, 1912; Neumann, 1929). Neumann, like Stieve, believed them to be wandering mesenchymal cells which ultimately reverted to connective tissue. Felix regarded them as primitive sex cells, which later degenerated, to be replaced by definitive cells from the coelomic epithelium. The others believed them to be, indeed, primitive sex cells, and progenitors of the definitive sex cells.

In the testis it is clear that many of these cells diminish in size and are incorporated into the sex cord; after which it is impossible to state their fate with finality. Although (through multiplication) they decrease in size, there is no evidence that they degenerate, as do those which fail to be incorporated. No such cells were ever found in the coelomic epithelium, and no forms transitional between them and coelomic cells could be found.

In the ovary, however, the primitive sex cells, already numerous in the indifferent stage, continue to proliferate actively until they form a large part of the organ. Mitotic figures occur everywhere. In older fetuses these cells enlarge, and the nuclear figures characteristic of germ cells are recognized. There is little difficulty in tracing the descent of these cells from the large cells of very early stages (6 to 8 mm.), because they become ever larger and more prominent. Later, when the fetal stroma invades the ovary, they may undergo massive degeneration, but the survivors are ultimately incorporated into follicles. At birth, and for months thereafter, there is no significant change in the structure of these follicles, and we must suppose that young Graafian follicles evolve directly from the primary follicles of the fetal ovary. The primitive sex cells of the indifferent gonad of the female can be followed until they become definitive ova. The pregranulosa cells, however, are formed steadily from the coelomic epithelium throughout the first half of gestation; and in some ovaries the epithelium is especially active at the stage when the stroma invades the cortex.

In the female, therefore, the presumptive sex cells do not, at any stage, lose their identity, even temporarily. If, according to Stieve's interpretation, these cells in the male are merely modified connective-tissue cells, then they should disappear from the ovary in the same fashion, which is not the case. The evidence is very strong that they are true primitive sex cells; but if they do indeed degenerate in the male, then we are faced with the remarkable conclusion that the sex cells in the female have a different origin from those in the male—an untenable view. Since the cells in question appear in the genital rudiment before the coelomic ("germinal") epithelium forms sex cords, it is reasonable to maintain that this epithelium is not the primary source of sex cells. There is perhaps a possibility, not substantiated by this study, that some primitive sex cells enter the coelomic epithelium secondarily, and there give rise to sex cells from their new situation.

In conclusion, a study of the extensive material available supports the view that the coelomic epithelium does not form sex cells in man. The sex

cells enter the genital primordium from some extragenital source and eventually give rise to the definitive sex cells. No attempt was made in this study to discover the ultimate source, because unfortunately time was limited. In this respect this study fails to provide an answer to Hargitt's first question. On the other hand, in the female at least it has been possible to trace the primitive sex cells with reasonable certainty until they are incorporated into early Graafian follicles. In man, therefore, as in fish, reptiles, and birds, and in some mammals, the evidence indicates that the germ cells are segregated early in development. The primary function of the coelomic epithelium of the genital ridge is to provide granulosa cells in the ovary, and (probably) the Sertoli cells in the testis. If germ cells are formed postnatally in the ovary, which is unlikely, then they come in all probability from pre-existing sex cells. The lethal effects of the stroma, however, must be remembered; and if there is proliferation of sex cells after birth, we should expect to find some special provision for their protection. Oögonia without a capsule of granulosa cells, in the author's experience, have never been encountered in a postnatal ovary.

## Origin and History of the Granulosa Cells and the Stroma Ovarii

Having considered the origin of the germ cells, we now turn to other cellular elements of the ovary: the granulosa cells, theca cells, and stroma cells. The medullary cords and the rete ovarii will receive separate attention.

In the development of the ovary from the genital ridge, the coelomic epithelium, underlying mesenchyme, and mesonephros are closely related. This proximity of different tissues has been responsible in no small measure for division of opinion, amounting almost to confusion, concerning the origin of the constituents of the ovary. Broadly speaking, at least eight views have been put forward as giving the precise histogenesis of the ovarian constituents, as follows:

1. The entire ovary is derived exclusively from the coelomic epithelium (Schulin, 1881).

2. The coelomic epithelium contributes only the covering epithelium; the underlying mesenchyme gives rise to all other tissues except the germ cells (Beard, 1900; Fischel, 1930; De Burlet and De Ruiter, 1921).

3. The germ cells are formed from the germinal epithelium, the granulosa cells from the medullary cords (said to be derived from the Wolffian body), and the stroma from the mesenchyme (Kölliker, 1879; Waldeyer, 1870; Rouget, 1879; Mihálkovics, 1885; Allen, 1904).

4. The coelomic epithelium gives rise to germ cells and granulosa cells, and the mesenchyme contributes the stroma (Pflüger, 1863; Janosik, 1885; Winiwarter and Sainmont, 1909; Stieve, 1927).

5. The germ cells are derived from the coelomic epithelium, the granulosa cells and stroma from the mesenchyme (Felix, 1912).

6. The precise contributions of the various tissues are not sharply discriminated, but the ovary is developed from both the coelomic epithelium and the underlying mesenchyme (Van Vloeten, 1929; Grünwald, 1934, 1936).

7. The germ cells and some follicular cells are derived from the coelomic epithelium, the remainder of the follicular cells and all the stroma from the mesenchyme (Higuchi, 1932).

8. The follicular cells only are derived from the coelomic epithelium; the stroma comes from the mesenchyme and the germ cells come from an extragenital source (Swift, 1914).

Of all these views, that held by Kölliker, Rouget, and others is the least acceptable. They misunderstood the nature of the medullary cords, which are not derived from the Wolffian body, and do not contribute granulosa cells (Winiwarter and Sainmont, 1909; Allen, 1904; Felix, 1912).

Except for that of Beard, Fischel, and De Burlet and De Ruiter, other theories have one feature in common, namely, they accept the participation of the coelomic epithelium in gonad formation, but are at variance as to the nature of its contribution. Fischel emphatically declares that the primitive germ cells migrate into the mesenchyme of the genital primordium, whereupon the mesenchyme is stimulated to form cords around them, without exciting any reaction in the overlying epithelium. The granulosa cells, the stroma, and the rete elements are formed therefore from mesenchyme. Before proceeding further it is necessary to digress in order to consider whether the role of the coelomic epithelium is passive or whether it makes some contribution to the gonad.

If figures 1 to 6 are re-examined, it will be obvious that the coelomic epithelium is not inactive, but quite

the reverse. Mitotic figures are numerous, cells are invading the underlying mesenchyme, and in general it is different in all respects from the more regular epithelium found elsewhere over the mesonephros (fig. 2). In older embryos, crowding of the cells results in the development of cords. Short at first (fig. 3), the cords later are long and anastomose freely, still retaining continuity with the multilayered zone of cells derived from the simple coelomic epithelium (fig. 5). The cells of the epithelium and of the cords are indistinguishable; there can be scarcely any doubt that the cells of these cords came from the surface epithelium. From figure 6 alone (an embryo advanced in development) the surface epithelium can be seen to be extremely active, constituting a denial of Fischel's contention. Mitotic figures are numerous, cell membranes are indistinct, nuclei are crowded despite the thinness of the section. From this evidence it is obvious that Fischel's claim that the mesenchyme of the genital primordium alone gives rise to the ovary must be rejected. Beard (describing the skate) is positive that the coelomic epithelium is inactive, and insists that "germinal epithelium does not exist." It may not exist in his material, but there can be no doubt that the coelomic epithelium in human embryos participates actively in the formation of the gonad.

The author is in agreement with that part of the Beard-Fischel theory which denies the participation of the *germinal epithelium* in the formation of germ cells; but, so far as human embryos are concerned, there can be little doubt that the coelomic epithelium does participate in the formation of the gonad. The explanation of tumors of the human ovary (Traut and Marchetti, 1940; Geist and Gains, 1938; Shaw, 1939) in terms of the Beard-Fischel theory is untenable. Rejection of the Beard-Fischel theory does not imply that the mesenchyme plays no part in the formation of the ovary. On the contrary, the mesenchyme makes a real contribution, if not to the degree that Fischel thought. Schulin (1881), Coert (1898), and others have maintained, on the other hand, that the thickened peritoneal epithelium forms a blastema from which the entire gonad is later fashioned; a view which is the direct antithesis of that held by Beard, Fischel, and De Burlet and De Ruiter.

That an important part of the ovary is formed from coelomic epithelium, there can be no doubt; just how much is still undecided. The ovarian stroma, however, appears to be derived from mesenchyme (according to Mihálkovics, 1885, in a variety of amniotes; Janosik, 1885, in the rabbit; Winiwarter and Sainmont, 1909, in the rabbit and cat; Kingsbury, 1914, in the cat; and Felix, 1912, Stieve, 1927, and Higuchi, 1932, in man).

In this study attention was drawn to the fact that the ovarian stroma differentiates late, being first seen in fetuses between 100 and 130 mm. It is foreshadowed as a local condensation around the blood vessels, rete, and epoöphoron, whence it spreads peripherally into the medulla (fig. 27), and finally occupies the whole ovary (figs. 28–30). If the stroma were formed from the coelomic epithelium, such a sequence could hardly occur. Moreover, mitotic figures in stroma cells appear first in the hilum and later in the medulla; and the ovary proper is invaded only after the oögonia have been encapsulated by granulosa cells. Stroma cells, as we have seen, exert a lethal effect on naked egg cells, and the sudden overrunning of the cortex by stroma leads to their degeneration (figs. 27, 28). The differentiation of stroma from the mesenchyme controverts the views of Schulin and Coert, and supports those of Van Vloeten and Grünwald, who held that the underlying mesenchyme makes a substantial contribution to the ovary. The contributions of Schulin on the one hand, and of Fischel and De Burlet and De Ruiter on the other, are important landmarks. Schulin in particular focused attention on the role of the coelomic epithelium, and influenced many who did not necessarily accept his view in its entirety. Like that of Fischel, his theory holds only in part, as far as the human ovary is concerned.

Fischel also made a contribution in emphasizing the importance of the concentration of mesenchymal cells beneath the coelomic epithelium, a feature seen by Allen (1904) and Willier (1927, 1939), but not stressed. It is consistently seen in human embryos of about 6 mm. (figs. 1–3), but is less conspicuous in embryos larger than 10 mm. (fig. 5), in which it is now reorganized into strands interdigitating with the sex cords. This early concentration of mesenchyme is no less a part of the genital primordium than is the coelomic epithelium, and Fischel considered it entirely responsible for the formation of the gonad. In later development it obviously be-

comes increasingly difficult to decide what is derived from the coelomic epithelium and what from mesenchyme. Higuchi, Van Vloeten, and Grünwald faced this dilemma and agreed that the rather heterogeneous mass of cells has a double origin. Grünwald in an 11.5-mm. human embryo found no clear line of division between surface cells and mesenchyme. In his scheme of histogenesis he carefully avoids any such discrimination, and represents the genital primordium as arising from an indeterminate zone between the deepest layer of the coelomic epithelium and the contiguous mesenchyme. If Fischel saw mainly the role of the mesenchyme, and Schulin and Coert the importance of the coelomic epithelium, Van Vloeten and Grünwald realized the close interrelation between the two. The observations recorded here reveal that the gonad, apart from the germ cells, is derived from elements of both. The exact contribution of each remains to be decided.

Thus far it has been shown that the germ cells apparently, and certainly the stroma cells, are not derived from the coelomic epithelium. The former appear very early, whereas the stroma enters the medulla only in specimens of 100 mm. or larger. There has been less dispute over the origin of the theca cells than over that of any other constituent of the ovary, ever since the descriptions of Tourneux (1879), Janosik (1885), and other early workers. They undoubtedly arise from the stroma, and the latter in turn from mesenchyme.

The only cell still to be accounted for is the granulosa or follicle cell. Pflüger (1863), Mihálkovics (1885), Winiwarter and Sainmont (1909), and others have stated that the granulosa cells and germ cells arise from a common stem cell, lodged in the coelomic epithelium of the genital primordium. The origin of the germ cells has been described, with the conclusion that there is no good evidence for this view. The primitive sex cells appear before the coelomic epithelium becomes active, maintain their identity throughout development (as far as the ovary is concerned), and do not appear ever to enter the coelomic epithelium. But even in the chick, entrance of the germ cells into the epithelium (Swift, 1914, 1915; D'Hollander, 1904) did not mean loss of identity. On the contrary, Swift traced their later history and found nothing to show that they form anything but sex cells. It has been shown here that the germ cells enter the sex cords only after the latter invade the mesenchyme. The cords themselves are indubitably formed from the coelomic epithelium.

In the ovary the sex cords are gradually disrupted by growth of the constituents, but the sex cells are still easily distinguishable (fig. 28). The smaller cord cells arise continuously from the coelomic epithelium until the embryo is well over 100 mm. in length. Ultimately they surround the sex cells and, as granulosa cells, become a distinct entity. Later, however, when the stroma appears, massive degeneration of oögonia may ensue, especially in the cortex. This is associated in turn with renewed proliferation by the coelomic epithelium, often far in excess of the requirements of the oögonia. These newly formed cells may be aggregated as alveoli, tubules, cords, or formless masses, the "Eiballen" of Meyer (1915) (figs. 31–34).

In sum, human germ cells apparently arise as such, outside the gonad primordium; the theca cell is a modified stroma cell, of mesenchymal origin; the granulosa cells of the primordial follicle alone, of the various cellular elements, arise from the coelomic epithelium. The several constituents thus arise independently, and each follows its own line of evolution. It seems most unlikely that the granulosa cell can evolve into a theca cell, and vice versa, as Schiller (1936), Fischel (1930), and others have supposed.

## Characteristics and Potentialities of the Granulosa Cell

The granulosa cell has a remarkable cycle, few cells in the body showing a like capacity for transformation. Arising from the coelomic epithelium, it is at first a typical embryonic mesothelial cell. Within the early sex cord it is small, with a prominent nucleus and a sharply defined nuclear membrane, but with an indefinite cell wall. The chromatin is evenly distributed, and one or two small but prominent nucleoli are present (figs. 4, 6). Cells of the rete cords have the same origin, an important point in understanding some types of ovarian tumors. Lumen formation in ovarian cords is a feature of late stages, and then is found only in the rete area. After the gonad becomes an ovary the pregranulosa cell enlarges slightly, the nucleus becomes more vesicular, and in the cortical region the cells are evenly spaced in relation to the

primitive sex cells (fig. 20). The pregranulosa cell then changes little from the 17-mm. to beyond the 100-mm. stage (compare figs. 20 and 26). Cell growth is responsible in part for the thickening of the originally slender sex cord.

As the stroma appears in the hilum, the pregranulosa cells undergo the first of many noteworthy transformations, assuming an extremely flat form as several collaborate to enclose an oögonium (figs. 27–29). These flattened cells, with hyperchromatic nuclei, are now almost indistinguishable from the invading stroma cells; and without a knowledge of early development, it would be hazardous to theorize on their origin. Felix made this error, as did Waldeyer, when they derived the granulosa cells from mesenchyme.

In the medulla, the granulosa cell changes little until the stroma has invaded the cortex (figs. 28, 29). As the cortex is infiltrated, the cells of the medullary primordial follicles enlarge slightly and begin to assume a cuboid shape. The nuclei are more vesicular, and mitotic figures are encountered. This enlargement occurs in all granulosa cells, whether they are related to follicles or not. Proliferation of the granulosa cells begins when the fetus measures 260 mm., or even later, leading to the formation of a true stratum granulosum. Fluid is then produced and the cells become swollen, giving the young follicle a characteristic appearance. Soon the follicular cavity appears and typical fetal follicles are formed.

In the cortex, granulosa cells forming follicles pass through the same stages as those more deeply situated; those not associated with oögonia form small dark clusters (fig. 31), the "Eiballen" of Meyer.

When the primordial follicle is surrounded by a thick granulosa, an important change occurs in the adjacent stroma cells, which enlarge, while the nucleus becomes centrally situated. The cytoplasm is neutrophilic, with fine basophilic granules clustered at one pole of the cell, as described previously for the interstitial cell of the testis (fig. 12). These are the theca cells, and are separated from the deepest layer of the granulosa by a delicate reticulum. At first there is a single layer, but later the developing theca may become thicker than the granulosa. When follicles undergo atresia (as they all do in the fetus) the granulosa cells are desquamated into the follicular cavity, and the theca cells enlarge and proliferate. The result is a mass of cells almost resembling granulosa lutein cells. In tangential sections such follicles suggest a corpus luteum in the fetal ovary, and call to mind the atretic follicles of pregnancy (Gillman and Stein, 1941). The theca cells are broken up into strands by the reticulum.

The granulosa cells in the numerous fetal ovaries examined did not actually luteinize, although Runge (1906) claims to have found a corpus luteum in a newborn infant. In the fully formed Graafian follicle the cells of the stratum granulosum are pear-shaped, sometimes even low columnar, resembling the corresponding layer in the preovulating follicle of *Elephantulus myurus jamesoni* (Van der Horst and Gillman, 1940, 1942). This is as far as granulosa cells evolve in the fetus.

Mention has been made of the fact that in some ovaries rapid intrusion of the stroma results in degeneration of many oögonia, and renewed proliferation of the coelomic epithelium. In some cases masses of pregranulosa cells result which are not associated with oögonia (figs. 31–34). When medullary and cortical follicles undergo enlargement (fig. 31), these cells participate in the process with the result that in parts of the cortex there may be clusters of granulosa cells organized as tubules, solid follicles (fig. 33), or alveoli (fig. 34). In some cases these structures are continuous with the coelomic epithelium (fig. 33). Their general appearance is very similar to that of the "Seminoma" described by Meyer. Such structures are hardly entitled to this inappropriate name, as the cells are essentially pregranulosa cells. However, the tubular structure often found in such cell aggregations is also of interest to gynecological pathologists. Tubules in ovarian tumors may indicate an arrhenoblastoma, or when well differentiated and not associated with tumor cells may simulate testicular rests in the ovary, as noted by Brambell (1930). Occasionally these masses of cells resemble the anovular follicles mentioned by Van Beneden (1880) and Gérard (1920) in the bat, by Regaud and Lacassagne (1913) in the rabbit, and by League and Hartman (1925) in the opossum. The latter believe that these anovular follicles may arise either by dissociation of the Valentine-Pflüger tubes, when no eggs are present, or by a disintegration of the ovum and persistence of the granulosa cells. If the egg is lost early, the follicle may survive for a long time, as is evidenced by the large

population of anovular follicles in which the granulosa is quite normal.

In the human fetus, aggregations of granulosa cells may persist long after birth, and according to Meyer (1915) may even form tumors. Geist and Gains (1938) declare that they have never seen such masses in the human ovary. These investigators must have been unfortunate in their material, since, in the author's experience, they are commonly found in the ovaries of young children. In the fetus they are usually associated with an extraordinary overgrowth of fibrous tissue (figs. 33, 34). The appearance of excessive fibrous tissue in the region of the tunica albuginea is itself noteworthy, as after puberty it may have serious consequences by impeding ovulation.

In connection with the fetal history of the granulosa cells one must again stress the fact that before the stroma invades the ovary (i.e. in fetuses of less than 100 mm.) the pregranulosa cells do not as a rule encapsulate the germ cells. At this time they are relatively large, with vesicular nuclei, whereas with the appearance of stroma they become greatly flattened as they encircle the oögonia. A multilayered stratum granulosum is invariably associated with differentiated theca cells, which thereafter are a constant feature of the follicle.

After puberty, when ovulation occurs, granulosa cells transform into lutein cells, which persist until menstruation supervenes. Thereafter they accumulate fat and cholesterol, and eventually atrophy and are replaced by fibrous tissue. In the corpus luteum of pregnancy they form numerous fine granules and later store colloidal material, some of which may undergo calcification (Gillman and Stein, 1941). Fat exists only as fine granules in the functional corpus of menstruation or of pregnancy. The presence of large quantities of fat or cholesterol is a sign of degeneration.

To summarize, the granulosa cells arise from the peritoneal epithelium covering the genital ridge, taking at first the form of cords with the characteristics of syncytia. Later, cell walls become better defined. Even in the cortex these cell masses represent part of the original sex cord. In this state they remain for a long period, until the invasion of the fetal stroma begins, when they break up to encapsulate the oögonia. As follicle cells they grow and proliferate, giving rise to the multilayered granulosa. At this stage a layer of theca cells differentiates from the stroma, outside the granulosa. The granulosa cells form fluid and later release it into the follicular cavity. Atresia of the follicle ensues, with desquamation of the granulosa cells and intense hyperplasia and hypertrophy of the theca cells. In circumstances where unencapsulated oögonia degenerate, renewed proliferation of the coelomic epithelium is initiated, resulting in excessive formation of pregranulosa cells which take the form of spherical masses, alveoli, tubules, or cords. Such masses seemingly induce the formation of thick sheets of fibrous tissue. The rete cords and the granulosa cells have a common origin from the original sex cord. Theca cells differentiate only around a primordial follicle, after the granulosa becomes stratified; they proliferate and enlarge during atresia.

There is thus an interesting functional relation between the granulosa cells and those of the theca; a matter which will be examined in more detail.

## Stroma and Theca Cells

The stroma of the adult human ovary is much more abundant than that in lower animals, including other primates. This is the opinion of Simkins, and is here confirmed. In the adult the stroma cell is spindle-shaped, and so attenuated that it appears to consist largely of a much elongated nucleus containing prominent chromatin granules. This type of cell, arranged in whorls, comprises most of the adult gonad but does not occur in fetal ovaries. For this reason a sharp distinction has been made between the stroma of the fetus and that of the child, and especially of the adult. As we have seen, the appearance of the fetal stroma comes relatively late in development, when the fetus is over 100 mm. in length. It first appears around the vessels and rete tubules in the mesovarium, and only later enters the medulla, where it is preceded by encapsulation of the oögonia (fig. 27). The tissue then invades the cortex, first along the septa demarcating the primitive sex cords. These septa, ordinarily inconspicuous in young embryos, are brought into relief by the advent of the stroma (figs. 25, 27). The stroma does not permeate

the whole ovary until the fetus is well over 250 mm. in length, in fact in some cases not until some time after birth.

As the stroma extends along the septa it merges with the tunica albuginea, and in the region of contact a fibrous thickening occurs. A fold of the covering epithelium may become involved, and flat, cuboidal, or even columnar cells are seen to line a small cavity, which is surrounded by dense, fibrous tissue. Such cavities may lose communication with the surface, with the result that a large tubular structure is included in the cortex.

When the fetal stroma is first seen in the medulla, the cells are sparse and do not resemble mature cells. They are spindle-shaped, with slender extremities, but the nuclei are round or oval. They proliferate actively. Soon after formation of the primary follicles in the medulla, stroma cells begin to encircle individual follicles or groups of follicles, but there is no true theca until the granulosa cells become cubical or stratified.

While the oögonia are unencapsulated, the stroma, for a time at least, is confined to the septa, which may become disproportionately thick. In the superficial part of the cortex this sometimes results in a lack of stroma cells in some areas; but in typical ovaries, even in the cortex, oögonia are covered by granulosa cells and the stroma passes freely between the follicles. The appearance of the stroma unmistakably characterizes an ovary; it is a manifestation of femaleness no less than the primordial follicle.

Since this tissue is derived originally from the general mesenchyme, it has definite potentialities. It forms fibrous tissue very actively under certain conditions, especially in atretic follicles or when a corpus luteum is converted into a corpus albicans. Under other circumstances it may accumulate fat, although the cells may show little change. In edema, stroma cells are also highly modified. They become widely separated, the processes thicken, and when great numbers of such cells retain water the effect is striking. Edema is usually followed by fibrosis.

The most important role of the stroma is the production of theca cells, which sets in when the primordial follicles begin to accumulate fluid. Corner (1932) says significantly, "The cells of the theca interna are not of great cytological or physiological interest during the resting phases of the follicle, but during a brief period preceding maturation and during subsequent changes which result either in formation of a corpus luteum or in follicular atresia, these cells undergo important changes and no doubt take part in the physiological reactions which occur at this time." It would appear that the emergence of the theca cells depends upon some stimulus emanating from the follicle at a certain stage in its development; they do not appear except in association with a growing follicle. In the early phase of follicular growth the stroma cells form the theca externa, and only from those cells immediately related to the follicle. The author agrees with Corner that "whatever the origin of the theca interna, its cells do not differ in appearance from the fibroblastic type during the period of growth of the follicle." In the author's opinion, the fibroblastic type includes stroma cells.

The reaction of the theca in the fetal ovary is intense to a degree seen elsewhere only in the atretic follicles of pregnancy. The cells not only become very large, but multiply to form a zone of many layers. They may resemble miniature lutein cells of the granulosa. They are large, with the nucleus displaced slightly toward one pole; the cytoplasm is at first neutrophilic, but later contains many lipoid particles which degenerate into free cholesterol, neutral fats, and fatty acids. In atretic follicles they resemble in many respects large interstitial cells, but whereas the interstitial cell characterizes the early testis, that is, in fetuses of 54 to 140 mm., theca cells appear only when the embryo exceeds 245 mm. Both types are of course mesenchymal derivatives. When atresia is well under way the theca cell becomes fat-laden, and in ordinary preparations such cells appear highly vacuolated. Associated with this change is a fibrous reaction which especially involves the reticular membrane separating the theca from the stratum granulosum. Fibrous tissue is also formed outside the theca, so that for a while the cells are enclosed between two fibrous membranes. Later they are broken up into small clusters embedded in fibrous tissue.

It is important to remember that hyperplasia and hypertrophy of theca cells are normally followed by an intense fibrous reaction. This is not uncommon in tumors, as reported by Geist and Gains (1938). Fibrous tissue, however, also occurs in and around

those masses of granulosa cells proliferated from the coelomic epithelium late in fetal life. In brief, the stroma cell typically becomes a fibroblast, but in contact with a growing follicle becomes a theca cell. Later, during atresia or in the newly formed corpus luteum, theca cells in turn revert to fibrous tissue.

## Histogenesis of the Human Ovary in Relation to Ovarian Tumors

This rather long analysis of the histogenesis of the ovary is not without its purpose. Ovarian tumors are of all perhaps the most difficult to understand. Their classification is constantly under review, and even today the status of many is not clear. The value of embryology in interpreting these tumors has been much discredited, a view which reaches its apotheosis in the statement of P. Moulenguet, quoted by Varangot (1937) in his monograph on granulosa cell tumors (p. 29), as follows:

> La recherche des filiations entre les éléments embryologiques des glandes génitales et les tumoraux est intéressante en soi, mais il faut reconnaître qu'elle a apporté jusqu'ici beaucoup plus de conjectures que de démonstrations et que, d'ailleurs, on n'en peut tirer peu de conséquences pratiques. Il est donc loisible d'ignorer ces recherches sans dommage.

It is true that hitherto embryology has failed to provide a satisfactory answer to the many problems of the gynecological pathologist; and the contradiction between the findings of the pathologist and the clinical manifestation of the tumor have often been embarrassing. Morphology has its limitations, but frequently closer study of the apparent contradiction has revealed valuable facts previously overlooked or not correctly understood. If the histological appearance of a tumor cannot be accounted for in terms of the potentialities of any embryonic cell, obviously either the available data are inadequate, or else embryological studies are indeed of no use. In this dilemma a re-examination of histogenesis is desirable. Moreover, it commonly happens that a tumor, apparently of the granulosa cell type, has failed to give evidence of estrogenic activity. Often, in such cases, the diagnosis of granulosa cell tumors is made with reserve. Here the fault is not a lack of information on histogenesis, but rather on the physiology of granulosa cells throughout their life cycle. There can be little doubt that much of the present confusion regarding ovarian tumors is the result of erroneous interpretations of the origin and potentialities of the various cell types. Shaw (1939) accepts the Beard-Fischel theory and proceeds to reclassify ovarian tumors on this basis. Schiller (1936), however, favored Grünwald's view, and thus a new classification was introduced. A more accurate knowledge of histogenesis should serve to clarify the potentialities of at least some of the cells found in ovarian tumors.

The data presented in this study should also throw light on the range of differentiation encountered in a given tumor. Since granulosa cells have an entirely different origin from stroma cells, transformation of one into the other is theoretically unlikely. In view of the fact that at one stage in development granulosa cells and theca cells are closely associated, this association theoretically might occur also in tumors and need not be accounted for on the hypothesis that theca cells arise by a metaplasia of granulosa cells or a common stem cell.

As already described, the potentialities of the granulosa cell are many, and the structure of tumors arising from these cells may stem predominantly from one or more stages in their history. One may therefore expect cordlike masses with or without lumina, broad cell columns typical of the embryo or early fetus, flat cells characteristic of the early follicle, small cuboidal cells of the granulosa in its proliferative stage, columnar pear-shaped cells of the basal layer of the fluid-filled follicle, the luteinizing granulosa cell without fat, or the degenerating type with quantities of fat, etc. Then also, tumors structurally may take the form of alveoli, tubules, or solid masses, associated with much fibrous tissue (figs. 33, 34). All the theoretical possibilities enumerated are to be found in the magnificent collection of tumors in the care of Dr. Herbert Traut (of the Gynecology Department, Cornell Medical Center, New York), which it has been the author's privilege to study. Likewise, the numerous illustrations in Varangot's (1937) monograph, *Tumeurs de la granulosa,* bear testimony to the remarkable capacity of granulosa cells to recapitulate their developmental history.

Plate (1938) describes a tumor which he calls a gynandroblastoma because it is regarded as a com-

bination of a granulosa cell tumor and an arrhenoblastoma. His description of this tumor (p. 255) is of great importance in illustrating the potentialities of the granulosa cell:

> In section I, in a slightly edematous stroma, strands and islets of cells are seen having the appearance of granulosa cells. In the islets in many places a folliculoid structure can be strikingly seen. Section II shows a preponderance of the adenoma picture. The cavity is coated with a layer of cells resembling granulosa cells, and in its neighborhood strands of the same cells are seen. The solid strands merge gradually into tubules. Section III shows both constituents of the tumor along with each other. . . . One sees above a *moiré* pattern typical for a granulosa cell tumor and below the tubules characteristic of arrhenoblastoma. . . . In the growth elements of granulosa-cell tumor and of arrhenoblastoma were found. These components are seen completely separated from each other but a gradual transition is visible.

Because this tumor contained tubules it was regarded as an arrhenoblastoma; the presence of granulosa cells arranged in cords justified its classification as a granulosa cell tumor, and the combination resulted in the name "gynandroblastoma."

From what has been said concerning the potentialities of the granulosa cell, it is obvious that such a tumor might be anticipated (see also Meyer, 1930, and others). Tubules are no justification for regarding it as an arrhenoblastoma. True, they are preeminently a feature of the testis, but the coelomic epithelium of the ovary can also form cords and tubules. They may be homologized with a part of the seminiferous tubule, but that is no real reason why they should be regarded as masculine, and responsible for producing male sex hormone. The tumor described by Plate well illustrates the value of a knowledge of histogenesis in accounting for the variations encountered in ovarian tumors. Although it is not common to find all the potentialities of the granulosa cell realized in a single tumor, nevertheless two or more stages are frequently encountered, especially in the collections which Dr. Herbert Traut and Dr. Emil Novak kindly put at the writer's disposal. Similar variations have been described by Varangot in his excellent monograph.

Other aspects of this problem will be reserved for more detailed study at another time. The question of hormonal effects, and its bearing on the classification of granulosa cell tumors as gynandroblastomata or arrhenoblastomata, will be examined in the section on the physiology of the fetal ovary.

Theca tumors can be analyzed in the same manner. The theca cell, arising from the stroma cell, is ultimately a mesenchymal derivative. As such it is capable of forming masses of fibrous tissue, even in the normal ovary, as is seen in the corpus albicans. In theca tumors, too, overgrowth of fibrous tissue may be extensive, as Geist and Gains (1938) have shown. Actually some of the fibrous nodules encountered in the human ovary may be abortive theca cell tumors.

Theca cells normally appear only when the follicle is maturing, and in atresia may become rather numerous. Theoretically a tumor may begin as a hyperplasia of granulosa cells cytologically like those in the growing follicle. At this stage theca cells may be stimulated to differentiate from the adjacent stroma, and for a time at least the tumor may contain both elements. The granulosa cells, as in the atretic follicle, may then degenerate and at the same time extensive thecal hyperplasia may take place, the theca cell thus becoming predominant. Later, all granulosa cells may disappear, with only theca cells and the parent stroma cells surviving. In these circumstances there may also be a great increase in fibrous tissue, intermingled with patches of theca cells. Fibrous tissue may ultimately replace all cellular elements, persisting as the burnt-out remains of the original granulosa cell tumor. The relation between granulosa and theca cells in such tumors has already received the attention of Von Werdt (1914), Davanzo (1930), and especially Meyer (1915). Recently Traut and Marchetti (1940) have found that in the folliculoid type of granulosa cell tumor (constituting 20 per cent of their ovarian tumors) some theca cells also occur. In the young cylindroid granulosa tumor they describe a conglomerate mixture of theca and granulosa cells, a type also described by Greenhill and Greenblatt (1938). Later the degeneration of theca cells is accompanied by laying down of fibrous tissue and consequent disturbance in the arrangement of the surviving granulosa cells. Traut and Marchetti conclude

that most so-called granulosa cell tumors contain varying amounts of theca cells and that pure granulosa cell tumors are rare. Usually one-fourth to one-third of the differentiated cells are theca cells. In some tumors there was equal representation of the two types, and so-called theca tumors usually contained granulosa elements in varying proportions.

From this short review it is seen that the association between granulosa and theca cells in the embryonic ovary is duplicated in ovarian tumors. It must be emphasized again that this association by no means implies metamorphosis of one type into the other. Traut and Marchetti, however, hold that their evidence substantiates the thesis of Fischel and his school, who have maintained that all cells of the Graafian follicle develop from mesodermal elements. This question has been sufficiently debated, and the Fischel hypothesis shown to be untenable. The main point of this study lies in the demonstration that the granulosa cell, at a particular stage of development, induces the differentiation of theca cells from the stroma. The acceptance of this fact should do much to clarify the physiological relations of these cells in normal ovaries and in tumors.

## Histogenesis of the Testis

The histogenesis of the testis has aroused as much discussion as that of the ovary. The problems are much the same and the theories in vogue are similar. A tabulation of the various views which have been proposed is presented:

1. The coelomic epithelium gives rise to the entire sex gland (Schulin, 1881; Coert, 1898; Allen, 1904; Simkins, 1932).

2. The coelomic epithelium gives rise to the germ cells only, the tubules are derived from mesonephric elements, and the interstitial tissue from the mesenchyme (Waldeyer, 1887).

3. The mesenchyme alone is responsible for the formation of the entire testis (Fischel, 1930).

4. The coelomic epithelium provides the germ cells and the Sertoli cells; the mesenchyme adds the interstitial cells and reticulum (Mihálkovics, 1885; Nagel, 1889; Stieve, 1927).

5. The coelomic epithelium and the mesenchyme are jointly involved in genital development (Grünwald, 1936).

6. The germ cells are derived from the coelomic epithelium; the Sertoli cells are interstitial cells which have invaded the tubules secondarily (Bardeleben, 1897).

7. The germ cells are derived from some extragenital source; the coelomic epithelium forms the seminiferous cords (i.e. Sertoli cells), and the mesenchyme gives rise to interstitial cells (Swift, 1914; and the author).

No useful purpose would be served by discussing these various views in detail, for much of what has been said of the ovary applies equally to the testis. The two genital primordia, coelomic epithelium and the underlying compact layer of mesenchyme, have the same early history in both sexes. The coelomic epithelium forms the sex cords, the germ cells being incorporated into these cords secondarily, at an early stage of development (fig. 6). The mesenchyme contributes the supporting tissue. At a later stage when the mesenchymal primordium is broken up into narrow strands, it is naturally difficult to discriminate between these primordia.

As soon as differentiation sets in, much earlier for the testis than for the ovary, the sex cords are ensheathed by the surrounding connective tissue. The sheath, as well as the spindle-shaped cells of the intertubular spaces, is mesenchymal; but the deeper part of the cellular layer and the connective tissue are in intimate contact (fig. 9). In the cellular tunic the primitive germ cells may persist for a while after the tubules are completely separated from that layer, but they ultimately degenerate.

Although the sex cords appear early, they do not increase much in thickness during the first half of gestation. Immediately after the cords are formed, and after they have separated from the tunic, primitive sex cells may be recognized for a while in the cords; they rapidly lose their characteristic appearance, however, and come to resemble the other cells. In later stages, the germ cells again become more prominent (fig. 13). Throughout the latter half of pregnancy an increasing number of spermatogonia appear in the tubules, and they are still more numerous after birth. But, as in the ovary, a distinct phase of growth is initiated in the spermatogonia after fetuses reach a length of 120 mm.

## The Interstitial Cell

The origin of the interstitial cell has been a source of contention ever since Leydig's (1850) original description. It has been assumed that the interstitial cell is a modified plasma cell (Waldeyer, 1887), white blood cell, endothelial cell of a blood capillary (Čejka, 1923; Champy, 1913; Boll, 1871; Courier, 1922; Nonidez, 1920; Goodale, 1919). Bardeleben (1897) considered the interstitial cell to be a young Sertoli cell. Many investigators derive the interstitial cell from the coelomic epithelium without further specification (Lenhossék, 1897; Beissner, 1898; Nussbaum, 1880); others merely assert that it is not formed from the general mesenchyme (Allen, 1904; Whitehead, 1904). Simkins (1928) derived it and all other cellular constituents of the testis from the coelomic epithelium. One immediate precursor of the interstitial cell is said to be the genitaloid cell, a name introduced by Felix (1912). Others say that some cells of the sex cord transform into interstitial cells (Loisel, 1904; Benoit, 1923, 1929; Scheunig, 1923; Pellegrini, 1925). There are those who agree with Leydig that the interstitial cell is a specialized connective-tissue element (Kölliker, 1854; Plato, 1896; Maximow, 1899; Stieve, 1927; Esaki, 1928); whereas some older workers like Harvey (1875), and more recently Berger (1922), have even thought that the cells were sympathetic nerve cells. In short, this cell has been derived from almost every type of cell known to exist in the testis.

It has been shown here that the interstitial cells in the human embryo first appear as small spindle-shaped elements, lying between the newly formed seminiferous tubules. This intertubular tissue is derived primarily from the original mesenchymal primordium, supplemented later by ingrowth along the blood vessels from the mesenchyme of the mesonephros. The interstitial cells therefore are specialized connective-tissue cells; few if any come from the coelomic epithelium, as Allen, Whitehead, and Simkins affirm. No evidence could be found that these cells either are young Sertoli cells, or are derived from the sex cords; and they bear no resemblance to nerve cells, lymphocytes, or plasma cells. The author's view is that of Stieve, who states emphatically that they develop from mesenchyme, which is unrelated to the coelomic epithelium.

The interstitial cell of the testis is the homologue of the theca cell of the ovary. After its appearance in the intertubular tissue it shows no marked change until the fetus is a little over 30 mm. in length, when proliferation begins. Some cells then enlarge and show a well defined cell membrane. In a 54-mm. fetus the interstitial cell bed has become relatively enormous, and the growth of the testis seems to consist chiefly in a proliferation of interstitial cells. Differentiation begins in the most distal part of the gonad and spreads radially and circumferentially toward the hilum. Growth continues until the fetus is over 130 mm., when a peak is attained (figs. 11–13). At this stage some cells are enormous. They now form sheets or cords between the tubules, but already there is evidence of degeneration. In a relatively short time, when the fetus is 160 to 180 mm. in length, degeneration is advanced, and soon only edematous spaces are left between the widely separated tubules (compare figs. 14 and 16). Thereafter the tubules increase in complexity and encroach more and more on the space previously occupied by the interstitial cells. The surviving cells remain as small clusters, and at birth the interstitial tissue is at a minimum. Many investigators have noted that human interstitial cells are especially well developed in the very early stages of intra-uterine life (Mihálkovics, 1885; Hofmeister, 1872; Plato, 1896; Spangaro, 1901; Thaler, 1904; Branca and Basseta, 1907; Bouin and Ancel, 1903; Mita, 1914; Mott, 1919; Kitahara, 1923). Kasai (1908) stated that in the human testis they are most highly evolved between the fourth and fifth months, when they represent the most abundant tissue. Later they decrease greatly, and Branca and Basseta (1907) state that in the newborn infant they are difficult to find. Scheunig (1923) confirmed Kasai's observations.

A cycle in the development of the interstitial cells has been reported in many mammals. Allen (1904) mentions that the interstitial cells in the pig reach a maximum when the embryo measures 15 cm. (birth length 28 cm.), after which involution begins. Whitehead (1904) found two phases of growth, separated by a long phase of inactivity. The first is said to occur when the fetus is between 2.9 mm. and 3.5 mm., after which there is a phase of atrophy and reduction in size. The second growth phase is initiated at about 20 cm. and continues until near term. Bascom and Osterud (1927) did not find the two growth cycles of Whitehead, but reported instead that the interstitial

cells increase slowly until the fetus measures over 20 cm., when an acceleration occurs which continues until an age of 4 weeks. In a 12.1-cm. fetus the interstitial tissue comprises 40.1 per cent of the total volume of the testis, at 19 cm. it is 48.4 per cent, at 25.3 cm. 62.8 per cent, at 4 weeks after birth 69.2 per cent, and at a year 34.7 per cent. Bascom and Osterud say that at 20 cm. some factor powerfully accelerates the growth of the whole testis. In the rabbit, Allen (1904) finds that the interstitial cells degenerate 24 days after birth. Kudo (1922–23) studied the interstitial cells in the embryonic guinea pig. In a 2.7-cm. embryo they were not definitely present, whereas in the 4.7-cm. fetus they were numerous, although unevenly distributed; but at 5.7 to 6.5 cm. they were found in every part of the testis, and many contained a great deal of fat. At 6.8 to 7 cm., although still numerous, they were not found in such masses as in the previous stage. In the 10- to 20-cm. fetus, they were absent from the intertubular spaces and were present only where the cords were in contact with the tunica albuginea. Despite the fact that he found few interstitial cells in the testis at term, Kudo's conclusion is: "The decrease in numbers of the cells occurs distinctly in later embryonic stages. However, there is no actual decrease but only a relative decrease as a result of the rapid increase in size of the testis."

Cole, Hart, Lyons, and Catchpole (1933), in a careful piece of work on the gonads of the fetal horse, were impressed by the extraordinary rhythm of growth. At one stage, between 40 and 60 cm., the fetal ovary exceeded in size that of the mother. In an early stage of the testis (4- to 12-cm. fetus) islands of interstitial cells, resembling those of the ovary, were distributed uniformly through the gonad, whereas in the 45- to 65-cm. fetus the testis was almost a solid mass of interstitial cells among which were scattered testicular cords. At this stage the testis weighed between 50 and 60 gm. Thereafter a spectacular reduction occurs, and when the fetus is 90 to 100 cm. long, the gonad weighs 20 gm., that is, it has decreased to about one-third its weight in the earlier stage. The weight of the testis in the 100-cm. fetus is once more about that in a 25-cm. fetus.

It thus appears that in various mammals the interstitial cells hypertrophy and then atrophy during development. In the pig, atrophy does not occur until after birth; but in the guinea pig, horse, and man, it occurs considerably earlier.

During the first half of gestation, human interstitial cells grow much more rapidly and occupy a larger volume of the testis than the seminiferous tubules. In older embryos, however, there is a sharp reduction, with more active growth of the testicular cords. Toward the end of pregnancy, interstitial cells are only found irregularly, between the coiled tubules. There is thus a reciprocal relation between the growth and development of the interstitial cells and that of the seminiferous tubules. Moreover, the interstitial cells are at a maximum while the testis is lodged within the abdominal cavity. Once it is in the scrotum, they are sharply reduced, and even in postnatal life they do not regain their former volume. At puberty, the interstitial cells again increase, but cannot compare in relative volume with that found in the fetus of 100 mm.

In undescended testes, however, the interstitial tissue is virtually absent before puberty, but after puberty Vines (1935) was impressed by its great amount. In this case so great was the hyperplasia that some islets more than filled the low-power field of the microscope. This condition was associated with inhibition of spermatogenesis. The presence of sex inversion in these cases of undescended testis is of great importance, as we shall see. The period of interstitial cell hypertrophy in the fetus coincides with the differentiation of the accessory sex structures.

## The Rete Ovarii, Pflüger's Tubes, and the Medullary Cords

The rete complex is found in the gonads of both sexes. The main interest attaching to these tubules concerns their developmental origin. Pflüger's tubes are said to appear at an early stage in the development of the ovary and are regarded as peculiar to the female sex gland. The medullary cords of the ovary, however, are supposed to represent vestigial seminiferous tubules. The origin of the rete cords has been much disputed. To some extent the confusion arises out of imperfect knowledge of their histogenesis. Those who deny to the coelomic epithelium any role in gonad formation, equally reject any suggestion that

the rete tubules might be formed from that epithelium. Recently Burns (1941) has given a brief summary of the problem. The older investigators, Waldeyer (1870), Mihálkovics (1885), Janosik (1885), Winiwarter and Sainmont (1909), Winiwarter (1900), held that the glomerular capsules produced buds which increased in size and complexity to form the "organ of Mihálkovics." This complex later established continuity with the inner ends of the primary sex cords. Another view holds that the rete emerges from a condensation of mesenchyme in the hilar region. The cords thus fashioned communicate on the one hand with the ends of the sex cords, and on the other with the glomerular capsules (Firket, 1914; Swift, 1915; Wichmann, 1912). Those who derive the rete from the coelomic epithelium have two opinions as to the precise manner of its development. Felix (1912), Wilson (1926), De Burlet and De Ruiter (1921) consider that cells derived from the coelomic epithelium, lying in the deepest part of the gonad, form a blastema from which the rete tubules later emerge, to join the capsules of the Malpighian bodies. Allen (1904, 1906), Fraser (1919), and Burns (1941) trace the rete to a series of peritoneal funnels which appear first as cellular cords or slender canals arising from (or in continuity with) a thickened band of coelomic epithelium, covering the cranial part of the genital ridge. In the region of the hilum of the gonad they fuse or anastomose freely, while toward the mesonephros they radiate as discrete tubules which unite with individual glomerular capsules. According to Willier (1939), the evidence for a mesonephric origin of the rete is conclusive in lower forms, and Brambell (1930) also states that "the most general view, however, appears that it [the rete] is formed by downgrowth from the Malpighian corpuscle, the nephrostomial canals or the nephrostomes themselves." The origin of the rete testis, as determined in the human embryo, will be detailed after the status of the medullary cords and Pflüger's tubules has been examined.

The concept of the medullary cords was introduced by Waldeyer (1870), who stated that the seminiferous tubules were derived from the mesonephros, and that in the female these tubules remained vestigial, as the medullary cords. His view was later supported by Kölliker (1879) and by Balfour (1878). Janosik (1885) and Mihálkovics (1885) also agreed in principle with Waldeyer, but the former maintained that the coelomic epithelium proliferated once in the male and twice in the female. The first proliferation gave rise to the primary sex cords, which became seminiferous tubules in the male but remained rudimentary in the female as medullary cords; a second proliferation formed the ovarian cortex. Winiwarter and Sainmont (1909) accepted this concept (which may be called the classic theory) in general, but instead of two proliferations they described three. The first formed the medullary cords, the second was abortive, the third became the permanent cortex.

Felix (1912) realized that the coelomic epithelium participated actively in gonadogenesis, but denied the existence of sex cords. According to Felix, proliferation of the epithelium formed an unorganized mass of cells, the "epithelial nucleus." In the testis this mass soon separated from the epithelium, and the seminiferous cords gradually took form. In the ovary, Felix affirmed that the epithelial nucleus filled the entire space between the surface epithelium and the mesonephros. In the fetus of 50 to 80 mm. the epithelial nucleus assumed a looser structure in the region of the rete blastema, but remained compact peripherally as the neogenic zone. Felix adds, "no matter how the development of the neogenic zone occurs, cords are never formed; the zone always forms a single mass." Later he says:

> All the masses and cords in the region of the stroma [i.e. the hilum] are not destroyed by the tissue, some always persist and deserve the name of "cords" since they are completely closed, elongated and sometimes curved structures. They are always situated below the rete and therefore derived from portions of the epithelial nucleus. Accordingly, they are correctly termed "medullary cords." These eventually degenerate, the last disappearing towards the end of term.

Forbes (1942), in homologizing the seminiferous tubules with the medullary cords of the female, in general accepts the findings of Janosik and Mihálkovics, and the original interpretation of Waldeyer. He regards the first proliferation from the coelomic epithelium as forming the medullary cords, and the second the future cortex. The latter develops rapidly and crowds the remnants of the primary cords to a central position. As development progresses, and the cortex continues to develop, the medullary cords are said to disappear completely.

Willier (1939) also thinks that in the chick ovary two proliferations occur from the coelomic epithelium, the first forming the primary sex cords, and the second the permanent cortex. He concludes:

> In general, from the time of origin of the cortical sex cords, the primary cords cease to grow and undergo a retrogression which was formerly thought to be complete in birds and mammals. . . . In the embryo, after separation from the germinal epithelium, they undergo a transformation into the following forms: (1) distended tubules having a wide lumen and a low epithelium—germ cells, either isolated or in clusters, may appear in the lumina of such tubules, (2) clusters or cords of clear or "fat-laden" cells, (3) isolated medullary cord cells, (4) isolated primordial germ cells, (5) cords of primordial germ cells, (6) cords of primordial germ cells bound together by a basement membrane and (7) mixed cords consisting of germ cells and clear or "fat-laden" cells.

Willier is satisfied that the medullary cord is the homologue of the seminiferous tubule.

The concept of Pflüger's tubules implied that the surface epithelium was a true germinal epithelium which proliferated actively to form long cords. These extended into the prospective cortex through the underlying layer of connective tissue, the tunica albuginea. Later these cords or tubes were broken into clusters of cells, the egg nests. In the egg nests, some cells differentiated into ova, while other smaller cells formed a capsule, and thus the primary follicle was formed. Felix, however, denied the existence of Pflüger's tubes in the human ovary.

In reviewing the status of the medullary cords, Pflüger's tubes, and the rete testis, we find that no significant advance in the knowledge of these structures has taken place for almost seventy years. The medullary cords are still regarded as the female counterpart of the seminiferous tubules. From the descriptive part of this study there can no longer be any doubt that the coelomic epithelium does proliferate in human embryos to form cordlike masses. This process is foreshadowed in the 6.7-mm. embryo and is well advanced in the 10.6-mm. embryo, in which cords of cells form a complicated network (fig. 5). They are broadly continuous with the surface epithelium through the cellular layer, and the free ends of the cords, near the mesonephros, consist of smaller cells, the precursors of the rete.

The rete cords are at first more highly differentiated in the anterior region of the genital primordium, but there, as elsewhere, they are essentially sex cords and in no way different from those in the rest of the gonad. It is the deepest part of the cord, i.e. the first-formed segment, that becomes modified to form the rete. This observation supports the findings of Coert, Allen, Felix, and Wilson (*op. cit.*) that the rete is ultimately derived from the coelomic epithelium. There is no evidence that it forms exclusively in the cranial end of the genital ridge, to be shifted secondarily into the hilum of the gonad, as is the case in marsupials (Fraser, 1919; Burns, 1941); neither is there anything to suggest that "Mihálkovics' body" is ever formed from the mesonephros in man. The rete cords do ultimately connect with some of the glomerular capsules, but the mesonephric tubules alone form the vasa efferentia. In man, therefore, the rete seems to have a different origin from that in amphibia (Witschi, 1914) and birds (Firket, 1914; Willier, 1939, and others).

Though it was not difficult to follow the development of the rete complex, it was impossible to confirm the generally accepted view that "sex cords" are peculiar to the testis, and that in the ovary two (or possibly three) separate proliferations occur, as against one in the testis. Just prior to sex differentiation, the gonad has a certain basic structure. It consists of a thick outer cellular layer, continuous with a deeper zone of sex cords. The rete cords, and the sex cords in the region of continuity, are ensheathed by a delicate mesenchymal reticulum, which extends almost to the deepest part of the outer cellular layer. In this latter part primitive sex cells are present in considerable numbers. The indifferent gonad, whether it is to become ovary or testis, has apparently the same basic structure. Sex cords are a notable feature, and the outer cellular layer continues to add cells to the cords. The presence of discrete cords at this stage cannot be regarded as a male characteristic merely because such structures later become so prominent in the testis. Similarly, the outer cellular layer cannot be considered a purely female characteristic, the precursor of the neogenic zone. In the development of this indifferent gonad two distinct processes are involved, the production of the sex cords and the formation of the outer cellular layer; the two go on simultaneously, one does not precede the other.

As the indifferent gonad becomes a testis, the sex

cords become more compact and sharply set off, and the rete complex is better defined than before. The sex cords, however, retain contact with the outer cellular layer for a time. In the prospective ovary, the striking feature is the absence of any obvious specialization in that direction. The reticulum in the rete region is less prominent than in the testis, but the rete is continuous with broad sex cords which in turn merge with the cellular layer, as in the young testis. In the young ovary the sex cords are of looser structure and the interspaces smaller, and the outer cellular layer becomes gradually thicker, giving the impression that the cords have mysteriously disappeared. In fact they persist, in part as the rete ovarii and in part as the broad cords of the cortex. An outer cellular layer is present in the testis as in the ovary, but it forms ultimately only the primitive tunica albuginea (fig. 9). In the female it persists for months, until, with the invasion of the fetal stroma, it is reorganized. Using Felix' terminology, the neogenic zone is a structure common to the gonads of the two sexes no less than are the sex cords; but it continues for a longer time to add cells to the ovary.

Although the central mass in the young ovary may appear to conform to Felix' description of an unorganized epithelial nucleus, actually this is not the case. The formation of thick sex cords continues, although septa are not easily seen until the stroma differentiates. The masses of cells between the septa correspond to Pflüger's tubes, but are not formed in the way that Pflüger and others have thought. They represent the original sex cords, to which cells have been steadily added from the coelomic epithelium and by proliferation of primitive sex cells. Bad fixation, or post-mortem autolysis, often leads to an appearance simulating that described by Felix, with a solid outer zone in the cortex and a looser structure in the medulla.

It must be remembered that the form of the ovary causes vessels and cords to converge toward the hilum, where the rete cords are found and where the mesenchyme is denser than elsewhere. From the earliest stages the cords in this region are covered by a distinct reticulum extending for some distance toward the cortical area. The constituent cells are a mixture of germ cells and pregranulosa cells. Those segments nearest the rete complex correspond to the medullary cords, and as a rule are easily recognized, especially after the fetal stroma appears. This is due to two factors, namely, enlargement of the oögonia and reorganization of the granulosa. The contrast between medulla and cortex is now sharp (fig. 29). If the oögonia are few, or if they die, the granulosa cells persist as solid cords or even as tubules, as described by Willier in the chick; if they are numerous, however, they intermingle with pregranulosa cells (fig. 29). Sometimes the latter are sufficient to encapsulate all oögonia, and the medullary cords then break up suddenly. This event coincides with the invasion of the fetal stroma and is found in the 140-mm. fetus. Parts of medullary cords may persist as primordial follicles, as compact masses of small cells, as isolated pieces of cord, or even as tubules, depending on cell structure in the original cord and on the number of oögonia. The many forms which these remnants may take are not peculiar to the medulla; similar vestiges may be found in other parts of the ovary (figs. 33, 34).

The medullary cord, as we have seen, is merely the much narrowed segment of a continuous structure, the broad part of which lies in the cortex. It corresponds positionally to the straight tubule in the testis, and Kingsbury (1914) has actually homologized the straight tubule with the medullary cord of the ovary. There is no justification for separating the part from the whole in order to homologize medullary cord and seminiferous tubule. The potentialities of the medullary part are no different from those of the rest of the ovarian cord; that is, it may form primordial follicles, or it may persist in the various guises just described. Similar structures may occur in the cortex, accentuated by precisely the same factors that operate in the medullary region; that is, by invasion of the stroma and the growth of pregranulosa cells, preliminary to follicle formation. An excess of pregranulosa cells results in the peculiar formations shown in figures 33 and 34. Similar structures are sometimes found in the hilum.

The narrow cords of the hilar region are largely due to the radiate arrangement imposed as they converge and are brought into relief by the denser stroma. The rete cords are even more prominent and more tubular than are the medullary cords. Thus a gradient of differentiation exists, conditioned by the fact that the stroma appears first in the hilum and then spreads

peripherally. Even after the stroma is present throughout the ovary, it is more highly developed in the medulla than in the cortex.

In short, the structure of the indifferent gonad is in no way visibly affected by the genetic constitution of the sex. Both sexes have prominent cords, both have a thick outer cellular layer, both have a well differentiated rete blastema derived from the deepest extremity of the sex cord. Only after differentiation is advanced is it possible to identify the structures which have been named "Pflüger's tubes," which are in fact the enlarged sex cords, just before reorganization takes place. At this particular stage Pflüger's tubes are essentially peculiar to the ovary, but they are nothing more than the much thickened sex cords of the indifferent stage. Their later evolution into egg nests does not occur in the human ovary, nor do they arise in the manner described for other animals. That part of the cord which intervenes between the cortical segment and the rete represents the medullary cord of Waldeyer, Mihálkovics, Winiwarter, and others; and its fate is determined by the development of the stroma, and by the relative numbers of oögonia and pregranulosa cells composing it.

The fate of the sex cords of the indifferent gonad is determined by inherent differences in the two sexes. In the testis the cords transform into seminiferous tubules at an early stage. In the ovary, however, they are drastically reorganized to form primordial follicles, a process which, in contrast with that in the testis, occurs relatively late in development. Throughout the long growth period of the ovary the original cord structure is retained, although histologically inconspicuous.

## Physiological Considerations

In comparing directly the histogenesis of ovary and testis, it is impossible to ignore the physiological processes that underlie the metamorphosis of the indifferent primordium into the definitive gonad, especially in view of the spectacular changes in structure that occur so consistently at certain stages of development. These events are unquestionably manifestations of physiological activities initiated at still earlier periods. Only after demonstration of the basic facts of morphology is it profitable to direct attention to the dynamics of development.

The history of the fetal testis can be divided into five distinct phases which merge the one into the other. These are: (1) indifferent phase, (2) phase of sexual differentiation, (3) phase of interstitial cell differentiation and hyperplasia, (4) phase of interstitial cell atrophy, (5) phase of growth of the seminiferous tubules. Similarly in the ovary there are five periods: (1) indifferent phase, (2) phase of sexual differentiation, (3) phase of growth, (4) phase of fetal stroma and primary follicle formation, and (5) phase of Graafian follicle formation and of follicular atresia.

Once established, each of these phases represents an important landmark in the history of the gonad. Some are relatively short, as in the case of the indifferent phase; others, such as the growth phase in the ovary and the phase of interstitial cell hypertrophy in the testis, represent a considerable period in fetal life. During each phase, moreover, other events are happening elsewhere—in the ducts, the accessory glands, and the external genitalia. Disturbance of normal development may lead to abnormality, the extent of which is determined largely by the stage at which the aberration is initiated. Since the rhythms of activity in ovary and testis after puberty are generally admitted to be under endocrine control (and the vast quantity of data accumulated from experimental work justifies this view), it seems plausible that developmental processes may in some cases also be regulated by hormonal factors. Waddington (1940), in reviewing the problem of organizers, has emphasized the role of hormones in differentiation. The question then arises as to the part played by hormones in the development of ovary and testis. That estrogens and androgens profoundly modify the development of the gonads in many animals is indicated by the work of numerous investigators, as reviewed by Willier (1939) and Witschi (1939). Willier has summarized the effects in chick embryos. In genetically determined females, estrogen apparently has no important effect on the developing ovary; in the male, however, the left testis becomes an ovotestis under the influence of estrogen, in extreme cases almost indistinguishable from an ovary. The right testis, because of its special constitution, is less modified. Conversely, androgen transforms the right ovary into a testis, the medulla

developing typical sex cords; the left ovary also develops cords in the medullary zone, but the cortex becomes very thin.

In mammals, the first demonstration that hormones affect the development of the gonads came through the classic work of Lillie (1917) on the freemartin. Lillie brought out the fact that the freemartin is a modified female whose male partner is always normal, attributing this to the fact that hormone is produced much earlier in the male twin and thereafter affects the development of the female. No explanation is provided, however, for the early emergence of the testis. In the genetic male, therefore, estrogen is not generated in sufficient quantity to influence normal development. Similarly, in the normal female the androgen formed is insufficient to modify the ovary.

Normally the genetic factor is of paramount importance in determining sex. In chicks, apparently, it can be neutralized (temporarily at least) by hormones, but tends to oppose the hormone of the opposite sex. The problem is, where does the genetic factor operate? Does it act directly within the genital primordium or is it mediated by some other gland? If the primordia of all the organs appeared simultaneously and developed synchronously, we might presume that the genetic factor acted directly on the gonads and other sex primordia. But the gonadal primordia emerge relatively late, after many organs and tissues have differentiated to a considerable extent.

By the time the genital primordia appear, the adrenals have already formed. These were seen in a 5-mm. embryo. Uotila (1940) observed them for the first time in 6- to 7-mm. specimens. But the adrenals, in some cases, consisted essentially of closely packed spindle-shaped cells. Before the appearance of either adrenals or genital primordia, the liver cells are already well differentiated. The early development of the liver is rapid, and from its enormous dimensions it must obviously be important in the economy of the embryo and fetus. Recent work on the capacity of this organ to affect the metabolism of steroid hormones (G. R. and M. S. Biskind *et al.,* 1939–1943; Heller, 1940) suggests that its activities may be of significance during development. The mesonephros, of course, also appears early, but we cannot as yet directly associate this organ with sex differentiation, although the work of Korenchevsky (1939) and others indicates that the steroid hormones do affect the kidney, causing an increase in weight. The pituitary is rudimentary until development is well advanced. The primordium of Rathke's pouch is present in 3-mm. embryos, but its connection with the mouth is not lost until the 8th week, and it is only between the 12th and 16th weeks that it acquires its definitive structure. The thyroid, too, appears early (even before the suprarenal primordium), and in 23-mm. fetuses some follicles even contain colloid. The liver, thyroid, and adrenal therefore are all well developed organs by the time the indifferent gonad is about to differentiate. Only the first two, however, appear to be sufficiently developed to be capable of exerting an influence on the emergence of the gonads, and there is no evidence that they do. Such information can only be obtained experimentally.

However, from the appearance of the genital primordia until definitive differentiation of the gonad, important developments occur in the thyroid, in the liver, and especially in the adrenal, the cells of which are (excepting only those of the liver) the most highly differentiated in the fetus. The adrenals are large, ovoid structures, dorsomedial to the genital primordium; and in a 10-mm. embryo they have already lost contact with the coelomic epithelium. The cells are large, with faintly acidophilic cytoplasm and prominent nuclei. Blood vessels appear early, and in a 12-mm. embryo form a complex sinusoidal system, especially in the central part of the gland (Uotila, 1940). Thus the adrenal is highly differentiated before sex becomes manifest; a situation which cannot be neglected. It is possible, then, that the adrenal is involved in sex differentiation by the elaboration of hormones which act on the indifferent gonad. In none of the many embryos examined was sex clearly recognized before the adrenal became highly organized and vascularized. It is not necessary to emphasize the profound effects that the adrenals may exert later on sexuality, as repeatedly shown in cases of adrenal neoplasm in females. The possible role of the fetal adrenal as an intermediary in sex differentiation, especially in the early stages of the process, should not be overlooked in experimental work.

Once the definitive sex is established, secondary changes are initiated in the gonads, which in time affect accessory structures—the derivatives of the Wolffian and Müllerian ducts, the prostate, seminal vesicles, rete cords, etc. In the male, the rete cords are promi-

nent when the gonad is first recognizable as a testis. Then, after a latent period of a week or two, the interstitial cells differentiate and soon become as large as or larger than those of the adrenal cortex, initiating the phase of interstitial cell hypertrophy. This period parallels the growth phase in the ovary, in which the primitive sex cells divide actively, with a steady flow of cells from the coelomic epithelium and the outer cellular layer into the cortex. The early appearance of the interstitial cell is a noteworthy feature and supports Lillie's (1917) hypothesis that the sex hormone appears earlier in the male than in the female. This sequence of events is invariable in man. Experimentally, androgens can induce prostatic buds and many other changes in female embryos (Burns, 1939; Moore, 1941; and others). If androgens are formed chiefly in the testis, it is probably only after the organ is well differentiated.

The great hypertrophy of the interstitial cells in man and other mammals during fetal life has interested numerous investigators (Bascom and Osterud, 1927; Kudo, 1922–1923; Cole *et al.,* 1933). Bascom and Osterud were puzzled by the enormous quantity of interstitial cells in the fetal pig, for which they had no obvious explanation. Cole *et al.* (1933) found also in the horse that a tremendous development of the fetal gonads occurred after the disappearance of the gonad-stimulating hormone from the maternal blood, and the onset of regressive changes in the maternal gonads. They consider the great development of the maternal ovaries to be caused by the presence of the gonad-stimulating hormone, but conclude that the stimulus for the formation of the interstitial cell mass in the fetal gonad is not apparent and cannot be so explained. Selye, Collip, and Thompson (1933) have demonstrated in rats that within 12 days after hypophysectomy the interstitial cells show degenerative change. Evans and his coworkers (1936; Fraenkel-Conrat *et al.,* 1940) were able to restore atrophic interstitial cells by an extract of the anterior lobe of the hypophysis. In adult rats, therefore, it would appear that the integrity of the interstitial cell bed in the testis is associated with the activity of the pituitary gland. Recent work by Selye and Albert (1942) has shown that both estrogens and androgens are capable of suppressing the interstitial cells, but at the same time there is damage to seminiferous tubules. It is unlikely therefore that the androgens elaborated by the testis could lead to interstitial cell hyperplasia in the fetus.

When the interstitial cells begin to differentiate, the fetus is approximately 30 mm. in length, but maximal development is reached at 140 to 160 mm. In the 30-mm. stage the thyroid already has some follicles; but the hypophysis is relatively undifferentiated, as chromophile cell types do not appear until 12 to 16 weeks, at a length of 56 to 112 mm. It is possible that the fetal pituitary may function prior to cytological differentiation, but there is little information on its activity in man. In the pig, however, morphological differentiation appears to precede physiological activity. Nelson (1933) found basophils present in fetuses of 5 to 6 cm., with a marked increase at 7 cm., at which time a few eosinophils also make an appearance. In the 9- to 10-cm. fetus eosinophils are more numerous, but are distinctly outnumbered by basophils. The eosinophils increase steadily until the fetus measures 160 to 170 mm., when there is a regional distribution of the cells and the basophils are relatively fewer. By implanting equivalent amounts of the pig hypophysis, removed at different stages in development, into hypophysectomized tadpoles and mice, Rumph and Smith (1926) and Smith and Dortzbach (1929) found that growth-promoting hormone could be detected in the 7- to 9-cm. stage, but no detectable amount of gonad-stimulating hormone was present until the 17- to 18-cm. stage. The amount rose slightly in the 19- to 20-cm. stage, after which the increase was rapid. All agreed that the late manifestation of the hormone, in relation to the appearance of the cell types in the pituitary, might be attributable to the relatively crude method of testing.

The interstitial cells in the pig appear much earlier than the stage at which pituitary hormones have been experimentally detected. But due caution should be observed in interpreting these findings, since basophils are already present in the 5-cm. fetus. It is a strange coincidence, however, that when the interstitial cells hypertrophy in the testis of the 20-cm. pig, the gonadotrophic hormones can already be detected in considerable quantities in the pituitary.

In the human pituitary, chromophile cells can be seen for the first time in fetuses of 10 to 12 weeks, with a crown-rump measurement of 40 to 56 mm. The interstitial cells are already differentiating at 30 mm., so that it is likely that the pituitary factors

may appear in physiological quantities at this time. The later hyperplasia may be associated with accelerated activity of the pituitary body.

In the human adrenal also, important changes occur just before or at the beginning of interstitial cell hypertrophy in the testis. Vines (1935), with his ponceau-de-xylidene-fuchsin stain, was able to demonstrate in the fetal adrenal a red granular material in the cortical cells. In the male, fuchsinophil material was regularly found, from 9 to 20 weeks, in considerable amounts, without visible zonation; in the female, it appeared only from the 11th to the 15th week, with a maximum at the 14th, disappearing rapidly thereafter. Vines assumes that this material is in some way related to the presence of androgen, indicating a definite "male phase" in normal female fetuses. In the male, the appearance of fuchsinophil substance coincides with the differentiation of the interstitial cells; it is present throughout the phase of interstitial cell hypertrophy and then disappears.

At the 11th week (fetus of ±40 mm.) Keene and Hewer (1927) describe the development of the true adrenal cortex. The cortical cells multiply rapidly and are arranged around a central space. It is significant that in the female, the fuchsinophil material appears and the true cortex begins to develop just before the appearance of the fetal stroma in the ovary, i.e. toward the end of the growth phase. Throughout the growth period, when the "androgen phase" would be expected to prevail, fuchsinophil material is absent. In the ovary the stroma cells, as precursors of the theca cells, are the developmental homologues of the interstitial cells. The fact that fuchsinophil material appears in the adrenal cortex at the onset of stroma formation, and is most abundant in the male during the stage of interstitial cell hypertrophy, may be interpreted in a different way from that of Vines. This material may be the result of the activity of the interstitial cells, inhibiting the adrenal cortex, which then begins to degenerate. The fetal cortex, according to Keene and Hewer, does degenerate steadily after the 20th week, which coincides with the disappearance of the fuchsinophil material and the atrophy of the interstitial cells. In the male, there can be little doubt that the fuchsinophil material is found almost exclusively during the phase of interstitial cell hypertrophy. In 43-mm. embryos the prostatic buds have already appeared. In this sense the fuchsinophil material may reflect the androgenic phase. So far as the male is concerned, it is suggested that sexual differentiation is closely linked with the functional activity of the adrenal cortex. The pituitary begins to function, slowly at first and rapidly thereafter, leading to the great hypertrophy of the interstitial cells. This reacts on the fetal cortex by inhibition, and the fuchsinophil material is an expression of this inhibition. The steady involution of the fetal cortex, after about the 20th week, coincides with the atrophy of the interstitial cells and the emergence of the neocortex. The interstitial cells inhibit growth of the seminiferous tubules. Once the interstitial cells atrophy, the seminiferous tubules begin to grow rapidly.

In the ovary, however, during the long growth phase, when differentiation is in abeyance, fuchsinophil material would be expected to appear if its presence in any way indicated the androgenic phase of Vines. Differentiation of the stroma, however, occurs when the fuchsinophil material is present. This coincidence, as in the case of the male, suggests a definite alteration in adrenal activity, correlated with the reassertion of true ovarian differentiation, rather than its retardation. The absence of fuchsinophil material, therefore, suggests adrenal activity, which may suppress ovarian differentiation, either by a direct action or through the pituitary. The growth of ovarian follicles indicates pituitary activity, which might be attributed to maternal hormones; but Cole *et al.* (1933) showed in the mare that the fetal ovaries were large when the maternal ovaries were smallest. The same argument applies here. The presence of maternal hormones in early pregnancy is associated with the long growth phase in the ovary. Only when they have decreased sharply do follicles form. If maternal hormones are held responsible for follicular growth, then by the same token we must assume that interstitial cell hypertrophy is so explained. It seems strange that the maternal hormones should be impotent with respect to the ovary at a time when they are effective on the testis. The assumption that growth of the fetal follicles is due to maternal hormones is obviously unsatisfactory.

It must be remembered that the emergence of the permanent adrenal cortex is associated with atrophy of the interstitial cells in the male, and with the differentiative processes in the ovary. We have no information as to the relative rates of atrophy of the

fetal cortex, and the emergence of the permanent cortex, in male and female. Until this information is available, any explanation of the extraordinary differences in the development of the ovary and testis must remain speculative. There is strong evidence that the growth and subsequent degeneration of the fetal adrenal cortex is closely linked physiologically with the emergence of sex. We may say with Uotila (1940) "that the fetal cortex would seem to belong to that group of prenatal structures which Streeter (1938) would regard as 'temporary devices' with which some particular needs are met."

The gonadotrophic activity of the fetal pituitary differs in the two sexes. In the male it is manifested relatively early, if hyperplasia and hypertrophy of the interstitial cells can be regarded as an indication. In the female, follicular growth and atresia occur only in the second half of fetal life. This unresponsiveness of the ovary may be related to the long interval between the differentiation of sex and the differentiation of the true ovary several months later. Smith and Engle (1927) and Selye, Collip, and Thompson (1933) drew attention to the unresponsiveness of the young animal to hormonal stimuli. Thus, in many young rats, the luteinizing hormone of the urine of pregnant women fails to elicit follicle maturation and corpus luteum formation but causes theca luteinization.

The pituitary can exert its effect only if the gonads are sufficiently mature to respond. The undeveloped character of the human ovary in the first half of gestation suggests an incapacity to react to gonadotrophic hormones. The relative ineffectiveness of the female pituitary may be determined by the same factors which retard ovarian differentiation. Nevertheless, the discrepancies in the time at which the pituitary induces recognizable gonadal effects in the two sexes is noteworthy; and an explanation must be sought for the extraordinary hypertrophy and atrophy of the interstitial cells in the male fetus.

Morphologically, the sudden differentiation of the fetal adrenal cortex suggests functional activity at an early stage. The pituitary exerts its action much later apparently, when it stimulates first the formation of the follicle and then atresia, with which are associated a hyperplasia of the theca cells. Interference with the normal function of the hypophysis results in a disturbance in the follicular derivatives. Hypophysectomy does not cause complete atrophy of the ovary; the Graafian follicles disappear, but the primordial follicles remain. By the same token, the pituitary can act only on a differentiated ovary. Malfunction of the adrenal, however, often leads to profound disturbance in the sexual constitution of the female, as Broster *et al.* (1938) have shown in cases of adrenal hyperplasia. The fetal adrenal appears to play a significant role in the sexual differentiation of the gonad and the expression of sex constitution, rather than in affecting the secretory activity of the gonad. In a sense, the adrenal produces a differentiative factor and the pituitary a secretory factor.

## Sex Inversion

Embryos of vertebrates have the potentiality of becoming either male or female, depending on alternative control mechanisms acting at certain stages. Normally, genetic factors direct sexual development, in part through the mediation of endocrine controls, among which we must consider the adrenal cortex. By degrees one sex becomes dominant. If the sexual bipotentiality of the organism is accepted, then theoretically, by applying a suitable stimulus at a critical period, the genetically determined sex may be repressed and the other allowed to become dominant. It is known from the work of Dantchakoff (1936), Willier (1939), Witschi (1939), Greene, Burrill, and Ivy (1939), Burns (1939), and Moore (1941) that the application of androgens to genetic females or estrogens to genetic males may cause widespread disturbances of normal development. Masculinizing hormones of the ovary can cause profound changes in the female, and adrenal hyperfunction may also have this effect (Broster *et al.*, 1938). In the male, however, in tumors of the adrenal or of the testis, a comparable result has so far been recorded but rarely. The sex constitution, therefore, is much more stable in the male than in the female.

Vines (1938) has critically analyzed the problem of sex inversion in man, examining the role of the adrenal in this process. He assumes the bisexuality of the soma and states that "the ultimate sex of the normal individual must be determined by the predominance of the one sex and the necessary repression of the

other into a state of latency, and clearly this repression in the normal case must be determined very early in development." The indifferent gonad has certainly the potentiality of both testis and ovary. The outer cellular layer, the sex cords, the rete are structures common to the two sexes. The cells of the sex cords in the female give rise ultimately to the granulosa cells, in the male to the Sertoli cells; the sex cells become either spermatogonia or oögonia; the mesenchyme in the testis gives rise to the interstitial cells, in the female it forms the stroma which provides the theca cells; the rete ovarii and the rete testis have the same origin from the deepest extremities of the sex cords. The indifferent stage is very short in the male; the assertion of the male structure is rapid and may occur in some embryos as small as 12 mm. in length.

Using the fuchsinophil material in the adrenal as an indicator of the "androgenic phase," Vines concludes that in the male this stage lasts from about the 8th or 10th week to the 20th, and is followed by an estrogenic phase. In the female the androgenic phase is more transitory, lying between the 11th and 15th weeks. Vines adds:

> There is seen to be some support for the theory of the essential bisexuality of the soma which has been the general basis of the explanation of conditions resulting in masculinization of the female. Leaving aside the question of the chromosomal bisexual potentiality of the fertilized ovum, the mechanism of secondary or endocrine differentiation also shows such features. In the female a period of androgenic influence occurs just after the time of differentiation of the female sex organs; it is rapidly terminated, but the bisexual element is more difficult to determine; there is no period of the estrogenic type during the first five months and it is not until the end of this time that an estrogenic period can be considered to occur. But this is outside the vital period of sex differentiation and it is probably for this reason that estrogenic or feminizing changes occur so rarely in the male at any time. The estrogenic factor is present and finds expression postnatally in the normal presence of estrin in the male urine and in the occasional cases of feminization.

In this light let us re-examine the developmental changes in the fetal gonads and accessory sexual organs. We must first make an assumption (which is justified by experimental evidence) that the growth of the Müllerian duct system is dependent on the presence of estrogen, and that suppression of its growth may be due to the absence of estrogen or the presence of androgen. Similarly, the growth of the male duct system is due to the presence of androgens or an excess of androgens over estrogens, and its inhibition to the absence of androgens or a predominance of estrogens.

Even before the earliest primordia of the gonad appear, the mesonephric duct system is already present and has established contact with the urogenital sinus. Its early development and persistence are at first independent of sex, although it later gives rise to the male duct system. When the testes are first recognizable, Müllerian ducts are also present as small invaginations of the coelomic epithelium at the cranial ends of the mesonephroi, lateral to the Wolffian ducts. They appear in the 11-mm. embryo (Koff, 1933) or even in the 8-mm. embryo (Hunter, 1930), and therefore differentiate before sex is recognizable histologically. Development continues for a while even after the testis is formed, but growth is definitely less than in the female. As the interstitial cells differentiate, retrogression is accelerated, and by the time the male embryo is 30 mm. in length the Müllerian ducts are becoming vestigial. At this stage hypertrophy of the interstitial cells is already initiated, but the prostate primordium does not appear until much later, when the embryo is 45 to 50 mm. long. Thus, retrogression of the Müllerian ducts begins a considerable time before the accessory glands have differentiated. Moreover, at this stage the rete is much larger in the male than in the female, since the rete cords are present and presumably respond earlier to hormonic stimuli than does the prostate. The fuchsinophil reaction appears in the adrenal only after the gonad has differentiated and after the Müllerian ducts have reached the cloacal region. During the phase of interstitial cell hypertrophy the fuchsinophil reaction in the adrenal remains intense. There is thus a close parallelism between the two phenomena. During this rather long period the prostate takes shape, the vesiculae seminales are present (though rudimentary), and the penis enlarges considerably as compared with the clitoris. The Müllerian ducts continue to grow but at a reduced rate.

Wilson (1926) attempted to ascertain the sex of the fetus from the configuration of the external genitalia, as checked against a histological examination of the gonads. The criteria used were based on those of Spaulding (1921), which to a large extent depend on

the length of the urethral groove. Wilson experienced no difficulty in determining the sex of fetuses over 52 mm., which period coincides with the phase of interstitial cell hypertrophy in the male. In every one of 15 specimens measuring 52 to 113 mm. he was able to substantiate the macroscopic diagnosis of sex. In 10 specimens between 41 and 50 mm. he made but one mistake, in an embryo of 50 mm. In this case the external genitalia were of the female type but the gonad was histologically a testis. In the next series, including 25 embryos of 31 to 41 mm., a wrong diagnosis was made 9 times. Each of the 9 cases had been diagnosed as a female from inspection of the external genitalia, and each proved to be a male. In the 21-mm. to 30-mm. group, 35 embryos were studied, of which 10 were wrongly diagnosed. The error was in the same direction, i.e. a supposed female was actually a male. It is significant, too, that Spaulding found a preponderance of females from 14 mm. to 50 mm., and his macroscopic diagnosis was not checked histologically. Wilson concludes:

> The fact would apparently indicate that in many instances the development and differentiation of the external genitalia are markedly retarded as compared with the development of the sex glands and particularly so in the male embryo. In only three instances did we find the error of diagnosis occurred in the reverse direction, that is that supposed males proved actually to be females. . . . While recognizing the frequency of retardation of development of the external genitalia in the male embryo one or two other possible factors should be mentioned. The possibility of external hermaphroditism cannot be ignored. However, it would seem unlikely that such a possibility could apply to as high a percentage of embryos as we found in our series.

Wilson, therefore, had much difficulty in recognizing the male genitals before the phase of interstitial cell hypertrophy became fully established. The early growth of the external genitalia was less marked in the male than in the female. The continued growth of the Müllerian ducts in 23- to 30-mm. embryos suggests that the estrogenic phase in the male may occur early rather than late in development, as stated by Vines; although in both sexes, at this early period in development, hormonal control might as yet not be well established.

The most serious malformation in the male genital tract must be related to interference with the normal phase of interstitial cell hypertrophy. This would manifest itself in continued growth of the Müllerian duct, partial or complete suppression of the Wolffian duct system, failure of the prostate and vesiculae seminales to differentiate, and a female type of external genitalia. The seminiferous cords, however, appear early, and it is possible that the gonad would then have the character of an ovotestis, i.e. seminiferous cords in association with a thick outer cellular layer (which normally becomes the tunica albuginea). If interstitial cell hypertrophy were disturbed after the 50-mm. stage, then the abnormality would be less profound. The prostatic buds have then already differentiated, the Müllerian ducts are largely suppressed, and the urethral groove is much reduced. The outer cellular layer of the testis is also markedly reduced, and the gonad would resemble a testis rather than an ovotestis, as in earlier stages. The later the onset of the abnormality, the more would the disturbance affect the external genitalia, while the testis would be least affected.

In the female, too, there is growth of the mesonephric duct system up to the 42- to 50-mm. stage. Involution then begins, and, with the exception of small fragments, it disappears from the genital cord (Koff, 1933). The Müllerian ducts grow steadily throughout early development, with no significant modifications until the fetus reaches a size of 42 mm. They fuse when the fetus is a little over 60 mm. in length, but growth is slow (Hunter, 1930, fig. 2). In a 142-mm. fetus the vaginal region shows rapid and irregular growth, beginning at the sinus end of the cord. At 151 mm. the whole length of the cord is involved, the cells being of the squamous type. The vaginal lumen first appears in the lower central end of the cord, and at 212 mm. it communicates with the uterine cavity through the cervical canal.

A great spurt in the development of the female genital organs occurs therefore after the 140-mm. stage, a week or two after the fuchsinophil material has disappeared from the adrenal cortex. This material was found by Vines in the female adrenal only between the 11th and 15th weeks; that is, when the fetus is between 45 mm. and 100 mm. During this period there is nothing striking to be seen in the ovary. Soon afterward, however, the fetal stroma begins to invade the medullary region. The first follicle was seen in a 185-mm. fetus, at a time which corre-

sponds to the stratification of epithelium in the vagina (Koff, 1933). This suggests that estrogenic action is a factor when the primordial follicles are being formed. The onset of follicular atresia is associated with massive desquamation in the vagina and a thinning of the epithelium.

As in the case of the Müllerian ducts in the male, the female Wolffian duct system grows rather slowly until the fetus is 50 mm. long; thereafter it is completely suppressed. The later growth and fusion of the caudal ends of the Müllerian ducts occur without significant change in the structure of the ovary; there is growth, but no active differentiation or formation of follicles. The growth phase of the ovary only partially coincides with the fuchsinophil phase in the adrenal. Differentiation occurs only after the fuchsinophil material disappears. If the fuchsinophil reaction were an expression of the androgenic phase, the Wolffian ducts should continue to develop, being sensitive to androgens; but they are suppressed before the appearance of fuchsinophil material, while the Müllerian ducts continue to grow steadily. The genitalia also grow slowly in the female, but after 50 mm. Wilson (1926) never failed to identify them correctly. Thus, during the androgenic phase of Vines neither internal nor external parts react as would be expected. Even the rete ovarii is reduced. The suppression of the male features in the female may be due either to the absence of androgens or to their relative ineffectiveness in the presence of considerable amounts of estrogen.

The one puzzling feature encountered in the female is the retarded differentiation of the ovary. Almost five months elapse before the fetal stroma appears and a primary follicle is formed. Throughout this long period its structure changes little. Then differentiation is suddenly accelerated, and within a relatively short time the ovary assumes its definitive character. This event follows even after the disappearance of the transitory fuchsinophil reaction, but it also coincides with the commencement of degeneration in the fetal adrenal cortex and the growth of the permanent cortex.

Whether this long latent period is an expression of the androgenic phase of Vines is not easy to say. The growth of the Müllerian ducts and the suppression of the Wolffian ducts suggest the existence of estrogen, or its dominance over androgen. If androgens are present, then the least that can be said is that the human ovary is much more sensitive to androgens than is the testis to estrogens. On the other hand, the fetal adrenal may play a different role in the male, or its ascendancy in the male may be established much earlier.

Theoretically at least, the long latent period of the ovary renders it more vulnerable to developmental abnormalities. The nature and extent of these abnormalities, as in the male, will depend largely upon the time they are initiated. The ovary will be more affected than the duct structures, but the ducts too may be deeply implicated, as the vagina forms rather late. If development is disturbed before the fetus is 140 mm. in length, the vagina is a cord of cells, and the uterus is small and V-shaped, with a relatively long, narrow cervix. The presence of androgens, although not to be regarded as fully established, might conceivably transform the broad female sex cords into narrow, compact cords of male type. Such modification of ovarian development might thus result in an immature testis, with perhaps a thick outer cellular layer as well. The prostate, in such case, might be absent and it might be difficult to say whether the genitals represented genetically a disturbance of male or of female development.

Female development is obviously more dependent upon normal activity of the adrenal than is male. The extraordinary virilizing effect of adrenal tumors in the female has been generally recognized. The ovary too is capable of secreting large quantities of androgens, as is seen in some theca or lutein cell tumors. The hormonal balance (between the androgens and the estrogens) in the female is apparently delicate; in the male it seems to be much more stable.

The dramatic disappearance of the interstitial cells in the male during the second half of gestation remains enigmatic, as does the great retardation in ovarian development in the first half of gestation. The elucidation of the problem of differentiation, and of sex inversion, depends greatly on the understanding of these extraordinary differences in the development of the gonads; and the efficiency of the fetal pituitary is presumably limited by their capacities or predispositions to respond. These preparatory changes in the gonads are, however, at first independent of the pituitary, although the adrenal may be intimately concerned. The adrenal goes through two main

phases of development: the rapid growth and evolution of the fetal cortex, followed by its atrophy and the emergence of the neocortex. The fetal cortex grows rapidly during the first half of gestation, i.e. contemporaneously with interstitial cell hypertrophy and the phase of ovarian growth; in the second half, the fetal cortex atrophies, likewise the interstitial cells of the testis, but ovarian differentiation is accelerated. These events are remarkable coincidences, and if not causally related nevertheless reflect profound modifications in the physiology of development. Any disturbance of the involved mechanism may result in widespread abnormalities in the genital tract.

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## Plate 1

Fig. 1. (Series 6508. C.-R. 6.7 mm.) Transverse section, ×240, showing the thickening of the coelomic epithelium and the condensation of the underlying mesenchyme; a layer deep to the cellular mesenchyme which is relatively acellular; a mitotic figure in the coelomic epithelium. Primitive sex cells (*psc*) are seen below the coelomic epithelium. *wd,* Wolffian duct.

Fig. 2. (Series 6507. C.-R. 8.2 mm.) Frontal section, ×240. A stage a little more advanced than that portrayed in figure 1. Mesenchymal tissue is concentrated deep to the coelomic epithelium; the coelomic epithelium is forming short buds into the underlying mesenchyme; a prominent primitive sex cell (*psc*) is seen well below the coelomic epithelium, and a cord from the coelomic epithelium curves around this enlarged cell; the mesenchyme between the genital ridge and the mesonephros is less cellular than in the region of the actively proliferating coelomic epithelium.

Fig. 3. (Series 6516. C.-R. 9.6 mm.) Longitudinal section through the genital ridge, ×240. The coelomic epithelium has formed short cords of cells of varying lengths; the mesenchymal tissue passes in between cords; there is an increase in the number of large primitive sex cells (*psc*) deep to the coelomic epithelium and outside the cords; the cells composing the cords are clustered together and cell walls are not readily distinguishable.

Fig. 4. (Series 6516. C.-R. 9.6 mm.) Detail from figure 3, ×800. Two large primitive sex cells (*psc*) with round vesicular nuclei; there are none in the coelomic epithelium; a mitotic figure is seen in the coelomic epithelium; cells from the coelomic epithelium are passing into the underlying mesenchyme; a branching genital cord (*sc*) appears to the left. The nuclei of these cords are more chromatic though smaller than those of the deeper mesenchyme. Mesenchymal cells (*mes*) are found between the genital cords, from which they are not easily distinguished in the figure.

Fig. 5. (Series 6524. C.-R. 10.6 mm.) Frontal section, ×240. There has been a great enlargement of the gonad. The genital cords are continuous in many places with the coelomic epithelium (*ce*). There are large primitive sex cells (*psc*) lying between the cords, which are more widely separated from one another near the coelomic epithelium than in the deeper part of the gonad. The "rete blastema" (*rb*) is already evident and is continuous with the primary sex cords. The mesenchyme (*mes*) is dense in the base of the gonad, as indicated in the figure. Blood corpuscles are also seen basally.

Fig. 6. (Series 6524. C.-R. 10.6 mm.) Detail from figure 5, lower rectangle, ×800. There are many sex cells lying between cords, varying considerably in size, but all having the same general structure. The cells of the primary sex cords (*sc*) are small, with hyperchromatic nuclei. The coelomic epithelium (*ce*) is irregular and in parts is continuous with the primary sex cords. The mesenchymal cells (*mes*) lie between the cords and are not clearly shown except near one of the large sex cells (*psc*) as indicated in the figure; they are small, and paler than the other cells in the gonad.

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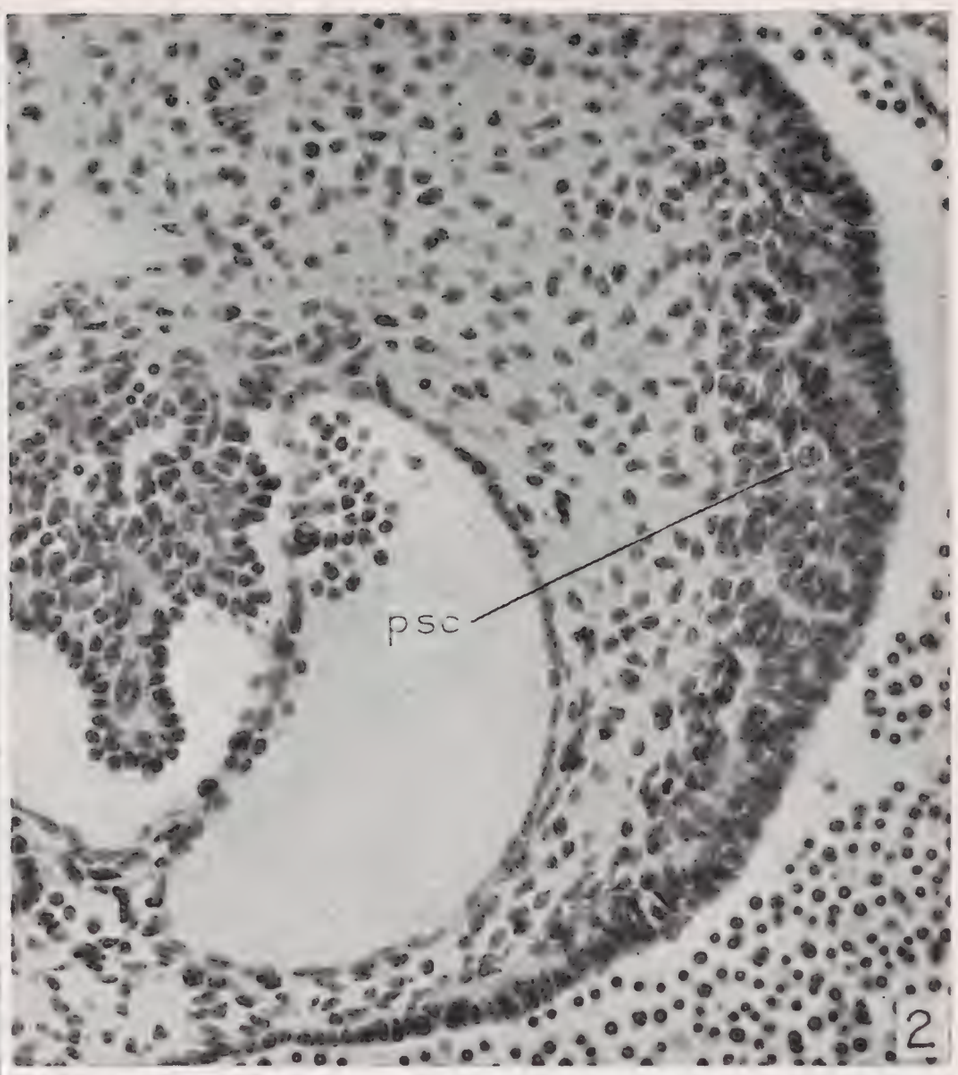
1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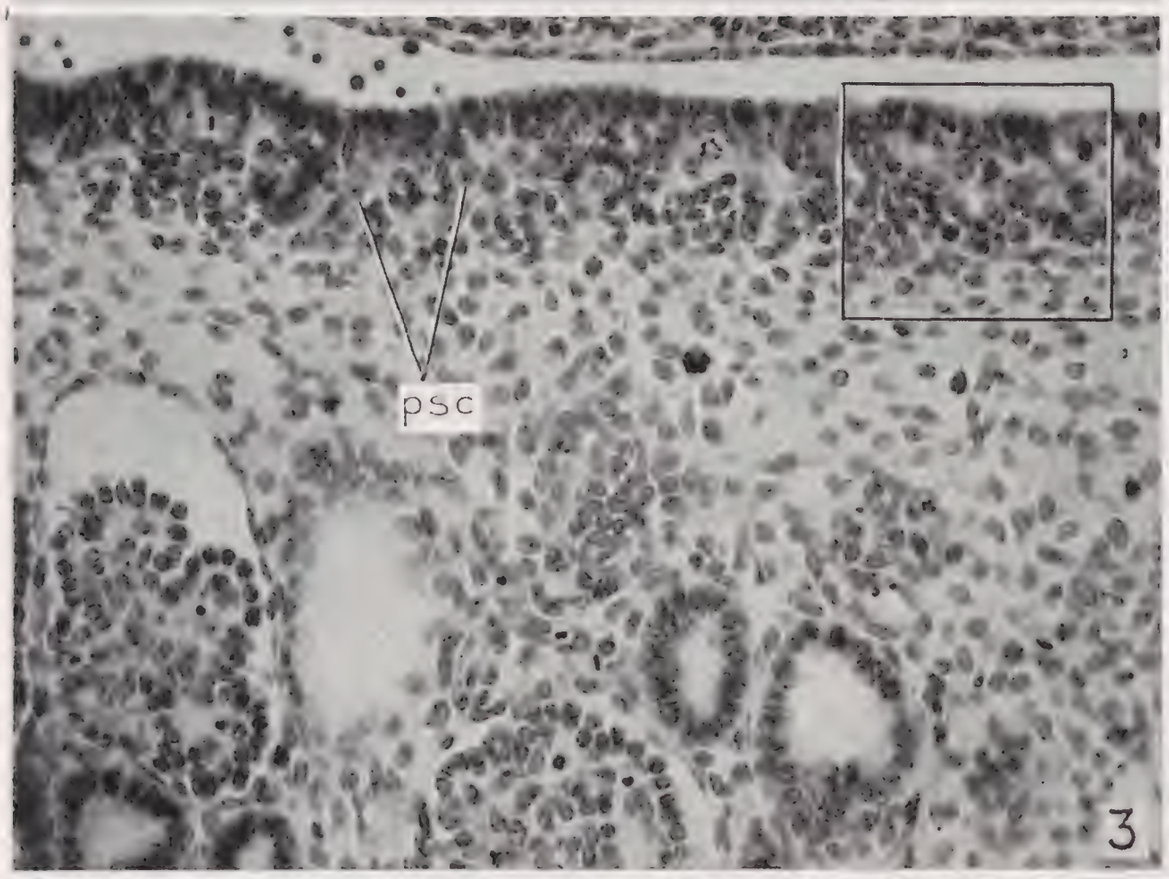
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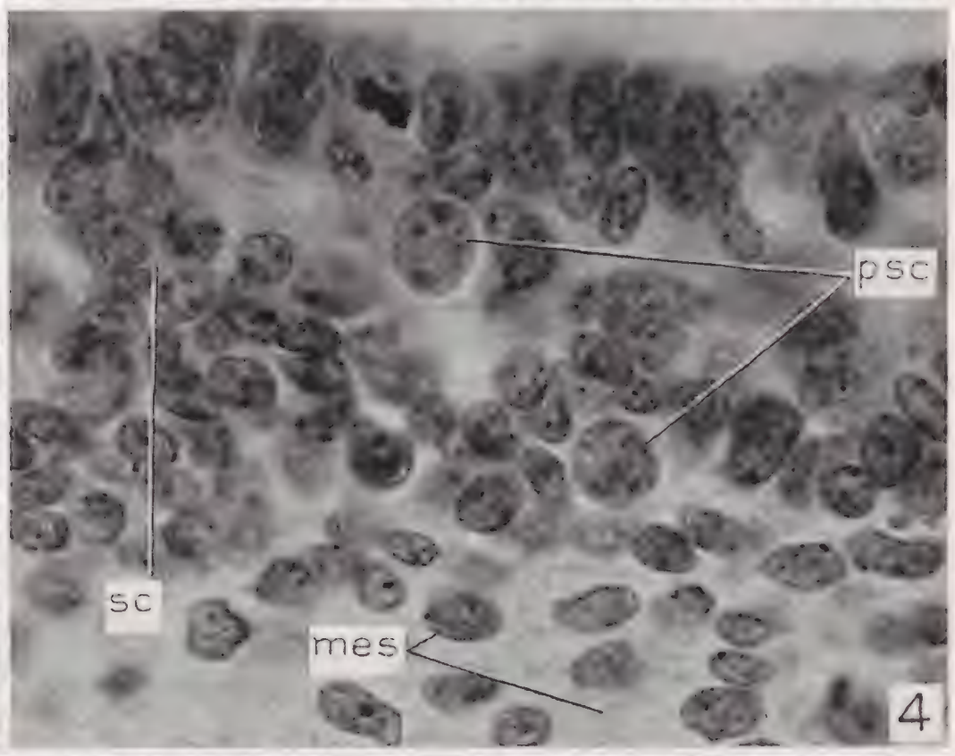
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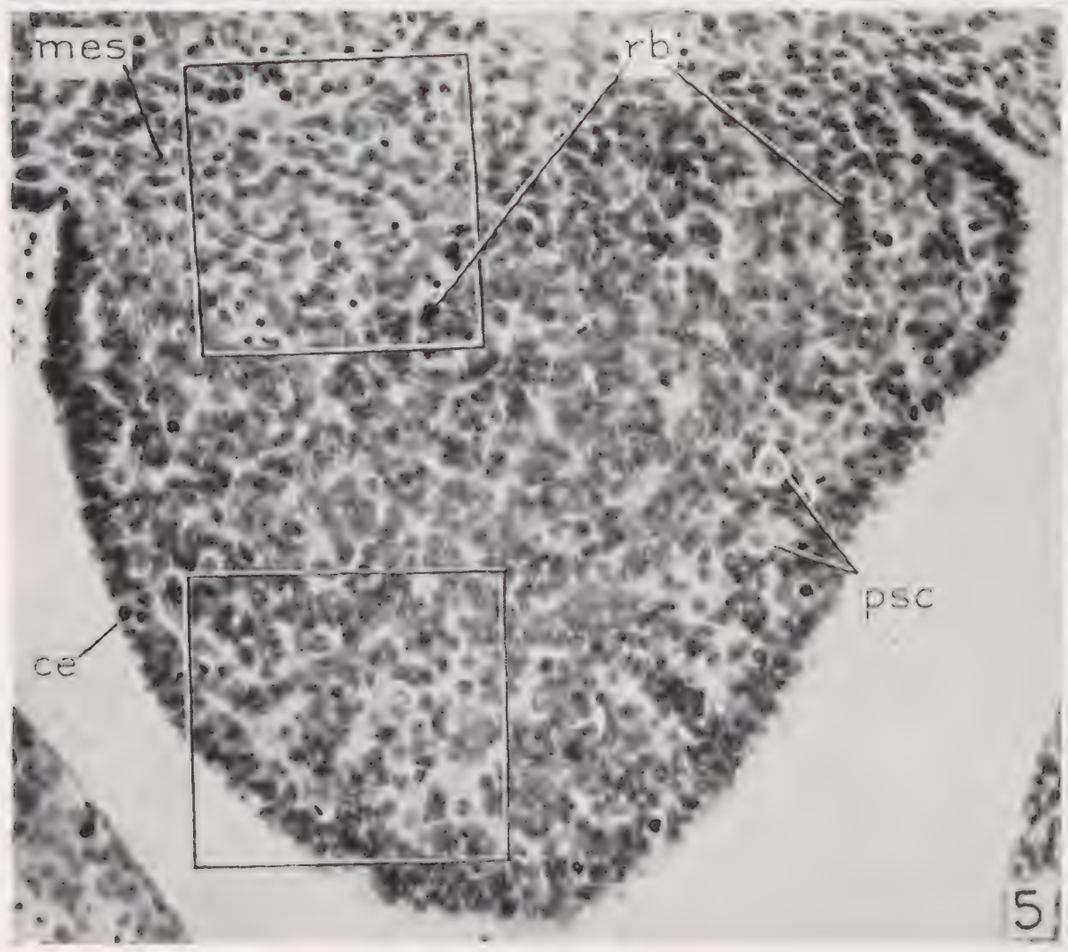
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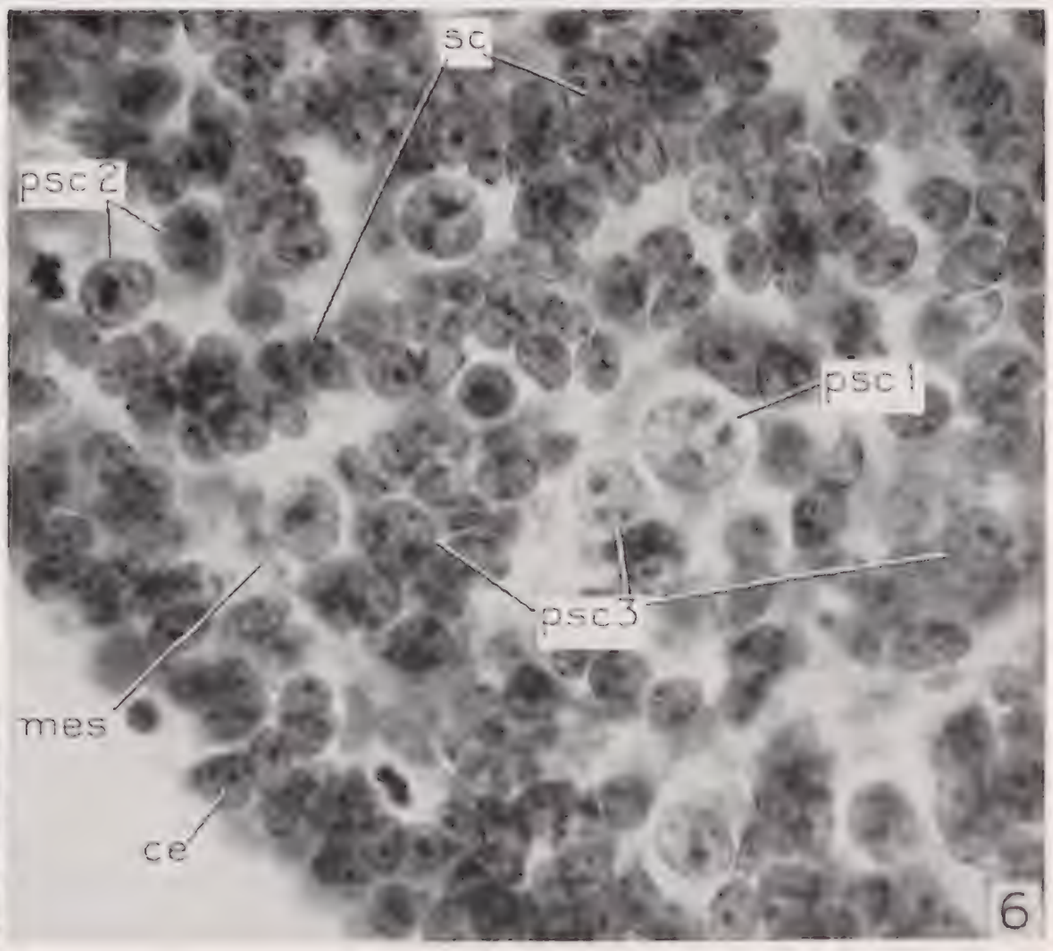
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FIG. 7. (Series 6524. C.-R. 10.6 mm.) Detail from figure 5, upper rectangle, ×800. This section passes through the rete region. A part of a glomerulus (*gl*) is shown in relation to rete cords (*rb*). Capillaries (*bc*) are found between the rete cords. Note the different appearance of the mesenchymal cells (*mes*) separating the rete cords from the glomerulus.

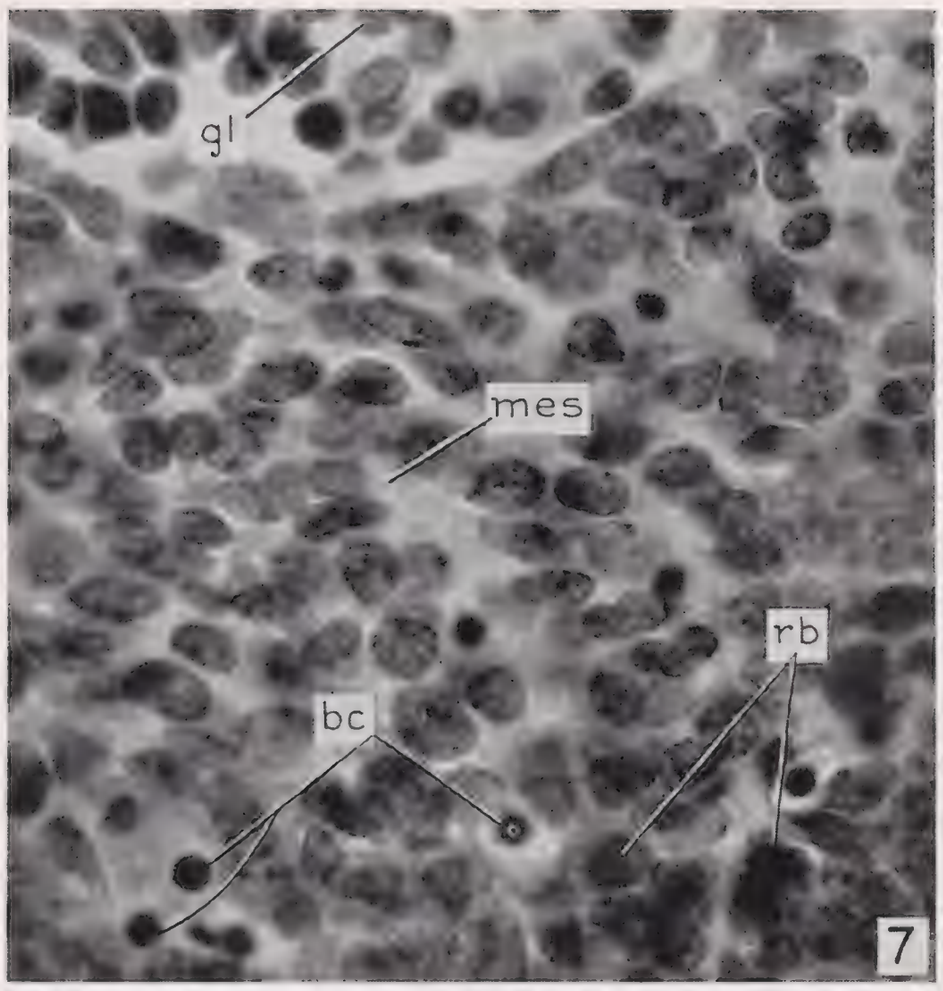
FIG. 8. (Series 5621 A. C.-R. 27 mm.) ×48. This section shows short, straight testicular tubules connected in parts with the rete testis; a well defined cellular tunic; and connective tissue growing in between the tubules into the substance of the testis. There is a clear zone between the darkly stained rete and tubules, which is of importance in connection with the problem of the fate of the primary sex cords in the female. *st,* straight tubules; *rc,* rete cords; *ta,* undifferentiated tunica albuginea.

FIG. 9. (Series 5621 A. C.-R. 27 mm.) Detail from figure 8, ×800. The coelomic epithelium is continuous with the tunic, which appears in turn to be continuous with the intertubular tissue; the testicular tubules are enclosed by a reticulum; two types of cell appear in the tubules. *tc,* testicular cords; *mes. s,* mesenchymal sheath of the testicular cords; *ce,* coelomic epithelium; *ta,* cellular tunica albuginea.

FIG. 10. (Series 3990. C.-R. 54 mm.) ×48. There is an enormous amount of interstitial tissue between the arcades formed by the testicular tubules. The interstitial cells begin to appear as a distinct entity when the embryo is about C.-R. 30 mm. and are well developed at this stage of the gonad. Note the thick tunica albuginea.

FIG. 11. (Series 1018. C.-R. 132 mm.) ×48. The testicular cords are now differentiated into convoluted testicular tubules and narrow straight tubules, with solid masses of interstitial cells lying between the tubules. The dark mass of tissue is the rete. Compare with figure 10. For area 1, see figure 12; for area 2, see figure 13.

FIG. 12. (Series 1018. C.-R. 132 mm.) Area 1, figure 11, ×800. There is a well defined tunica albuginea, with sharp demarcation between interstitial cells and tunic. The large interstitial cells resemble cells of the corpus luteum; connective-tissue cells surround the testicular tubule. *g,* granules in cytoplasm of an interstitial cell.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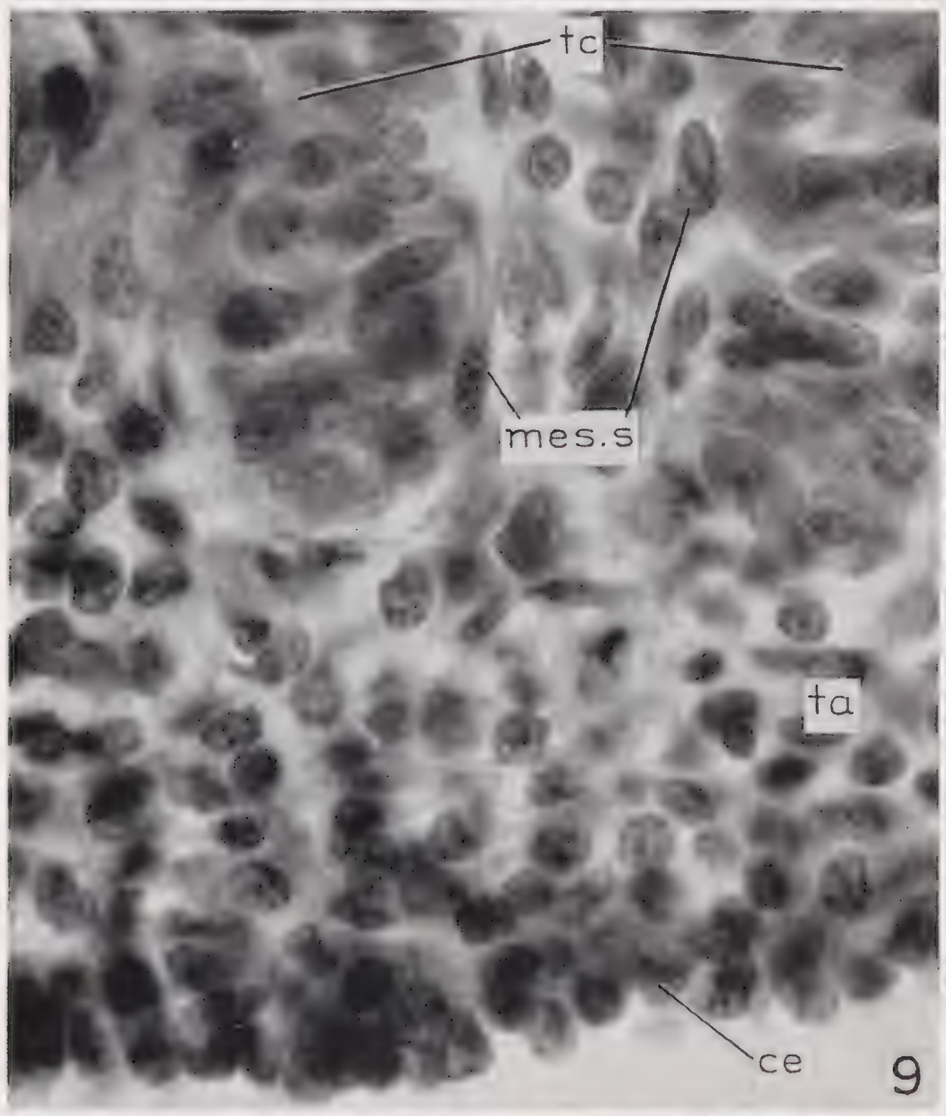
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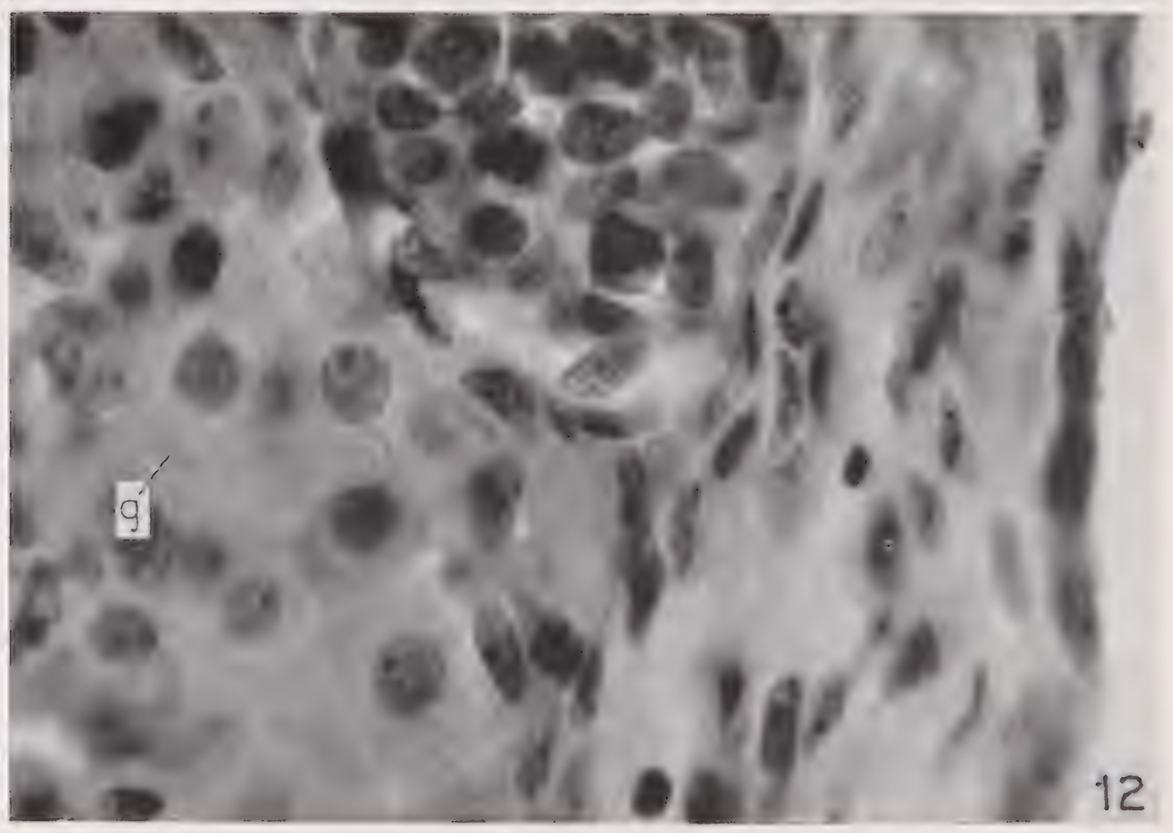
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FIG. 13. (Series 1018. C.-R. 132 mm.) Area 2, figure 11, ×800. Note the mass of interstitial cells, with well defined cell walls. Between the interstitial cells the endothelium of capillaries is evident. The large clear cell in the testicular tubule is probably a spermatogonium. This cell is distinctly different from interstitial cells. Note the cellular sheath of the testicular tubule.

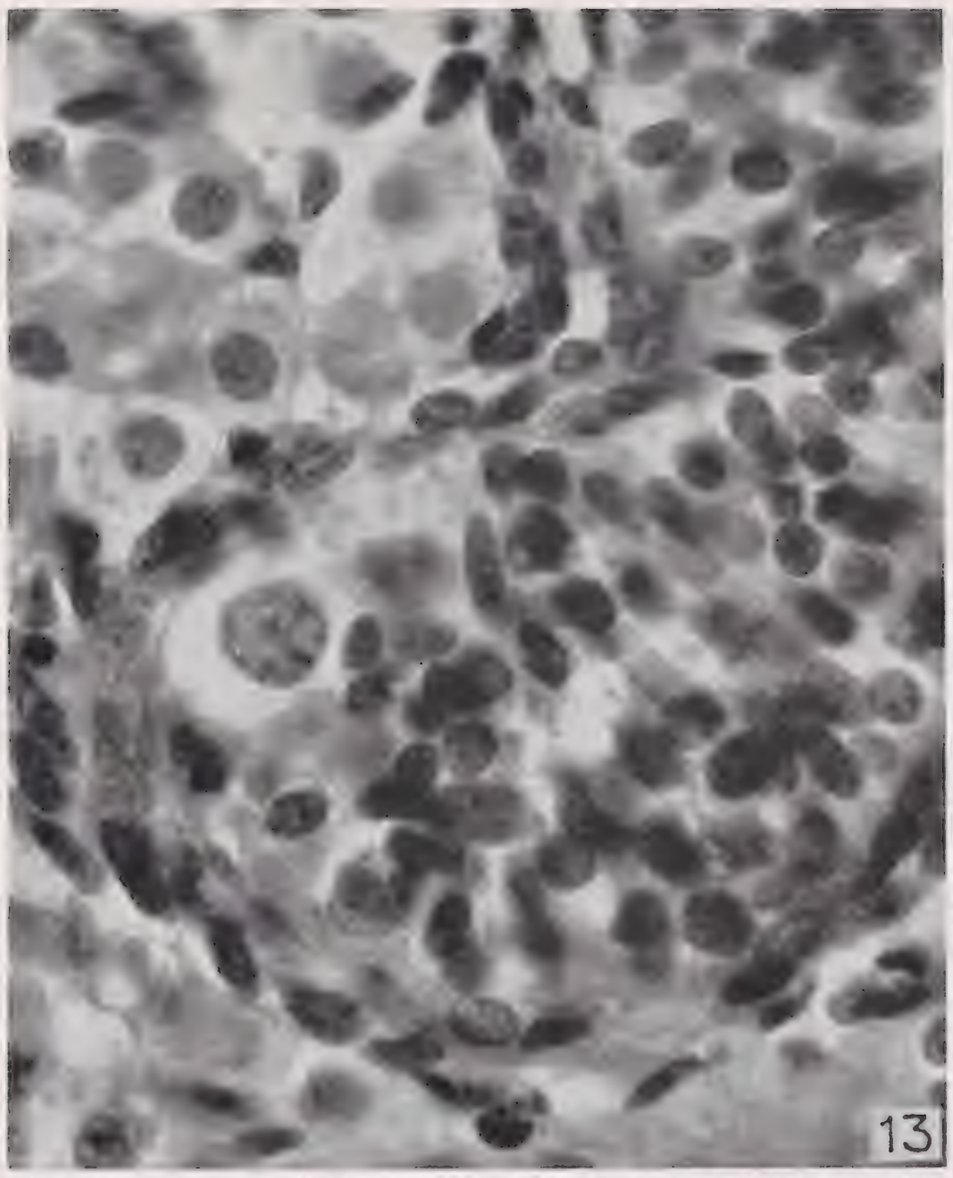
FIG. 14. (Series 1067. C.-R. 187 mm.) ×48. At this stage note the enormous mass of interstitial tissue; the coiled testicular tubules; the well defined tunic containing spermatic vessels. The interstitial tissue reaches its peak of development between 160 and 190 mm. C.-R., after which it atrophies very rapidly. Even in this photograph there is some evidence of atrophy.

FIG. 15. (Series 1067. C.-R. 187 mm.) Detail from the area outlined in figure 14, ×800. Note large groups of interstitial cells, which vary in size and staining reaction. Some are very vacuolated and in others the nuclei are almost pycnotic. These cells are beginning to undergo involution. In the adult testis, interstitial cells rarely exceed these in size.

FIG. 16. (Series 6679. C.-R. 286 mm.) ×48. This stage is remarkable for the disappearance of the interstitial cells; only patches of atrophic cells remain between the tubules. The disappearance occurs first in that part of the testis where they first differentiate. In the same testis, at the critical stage, it is possible to see masses of interstitial cells in one part of the testis and none elsewhere. Compare this figure with figure 14.

FIG. 17. (Series 6679. C.-R. 286 mm.) Detail from figure 16, ×800, showing atrophy of the interstitial cells; only a few large cells persist. These are at all stages between large cells and cells which resemble connective tissue (*ct*). The large cells in the testicular tubule are probably spermatogonia.

FIG. 18. (Series 4304. C.-R. 25 mm.) ×48. This view of the ovary at low power shows an ill defined rete (*rc*) and very cellular cortex. Compare it closely with the testis of figure 8. The area outlined is shown at higher magnification in figure 19.



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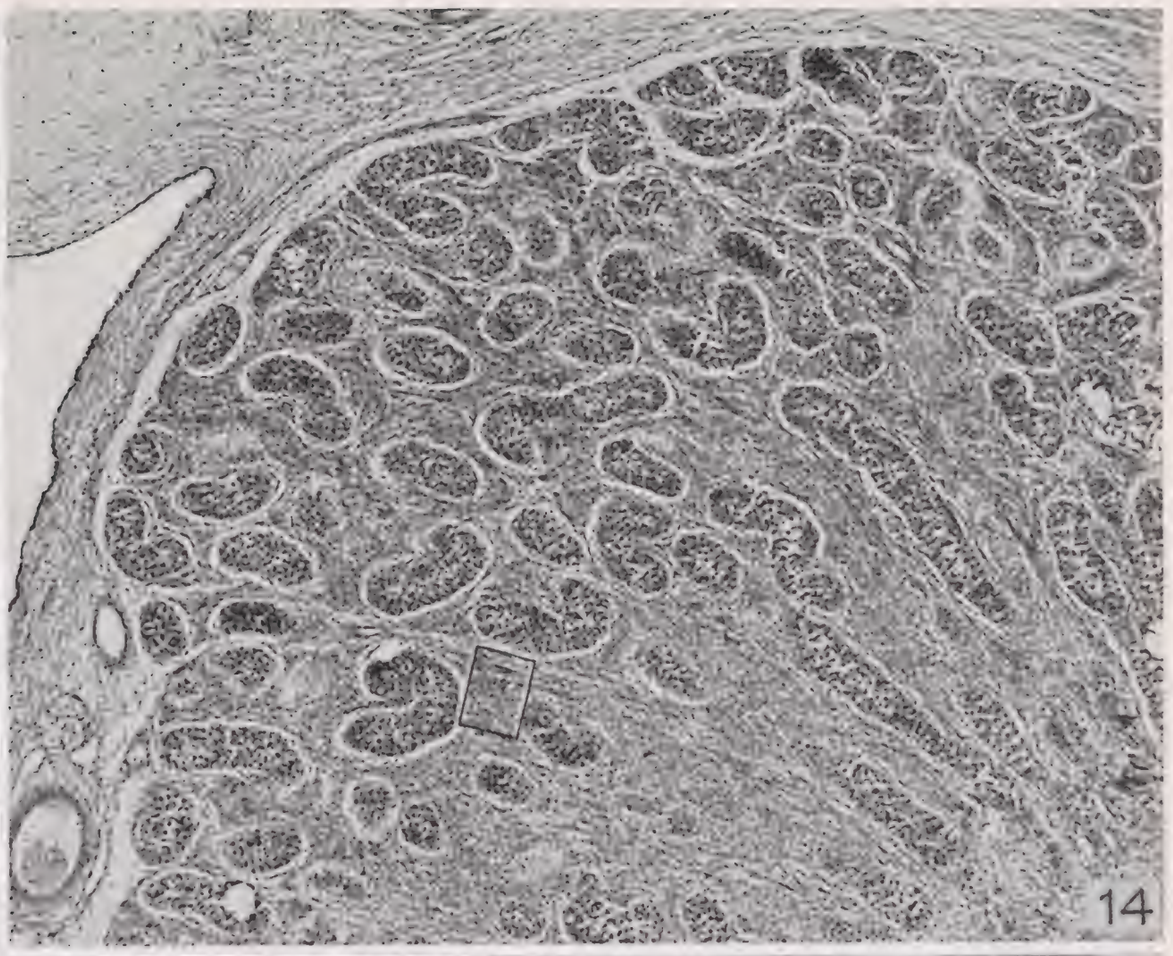

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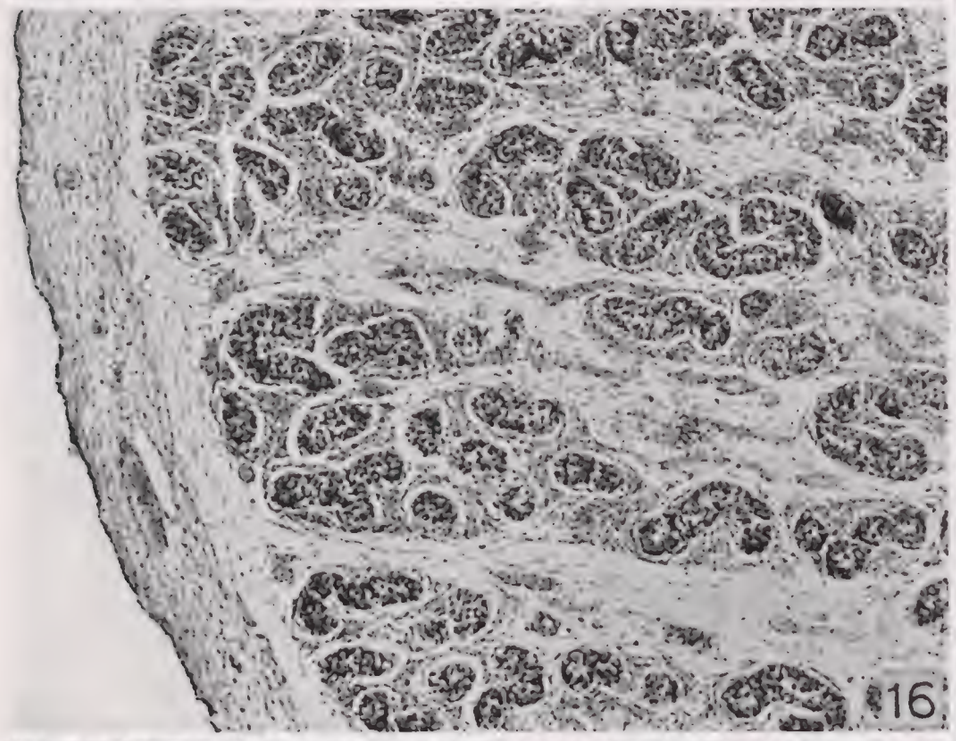

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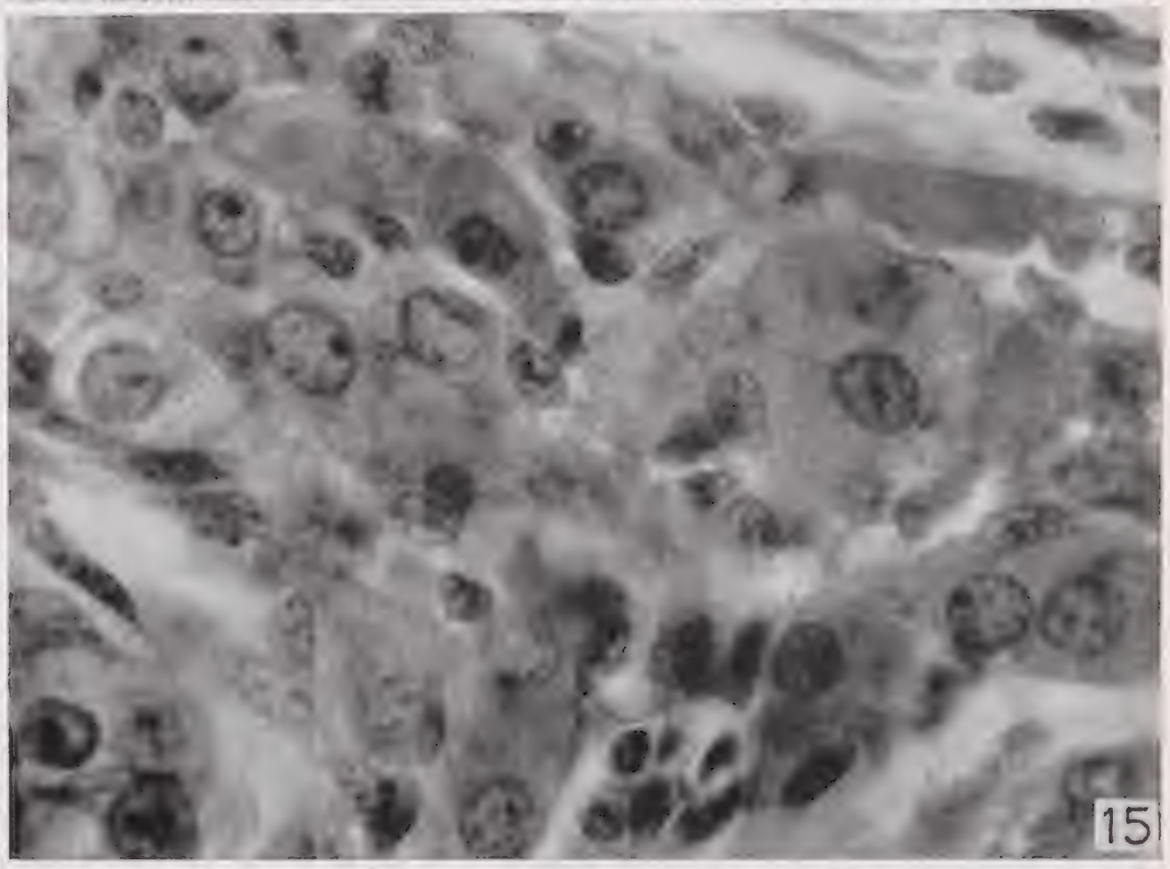

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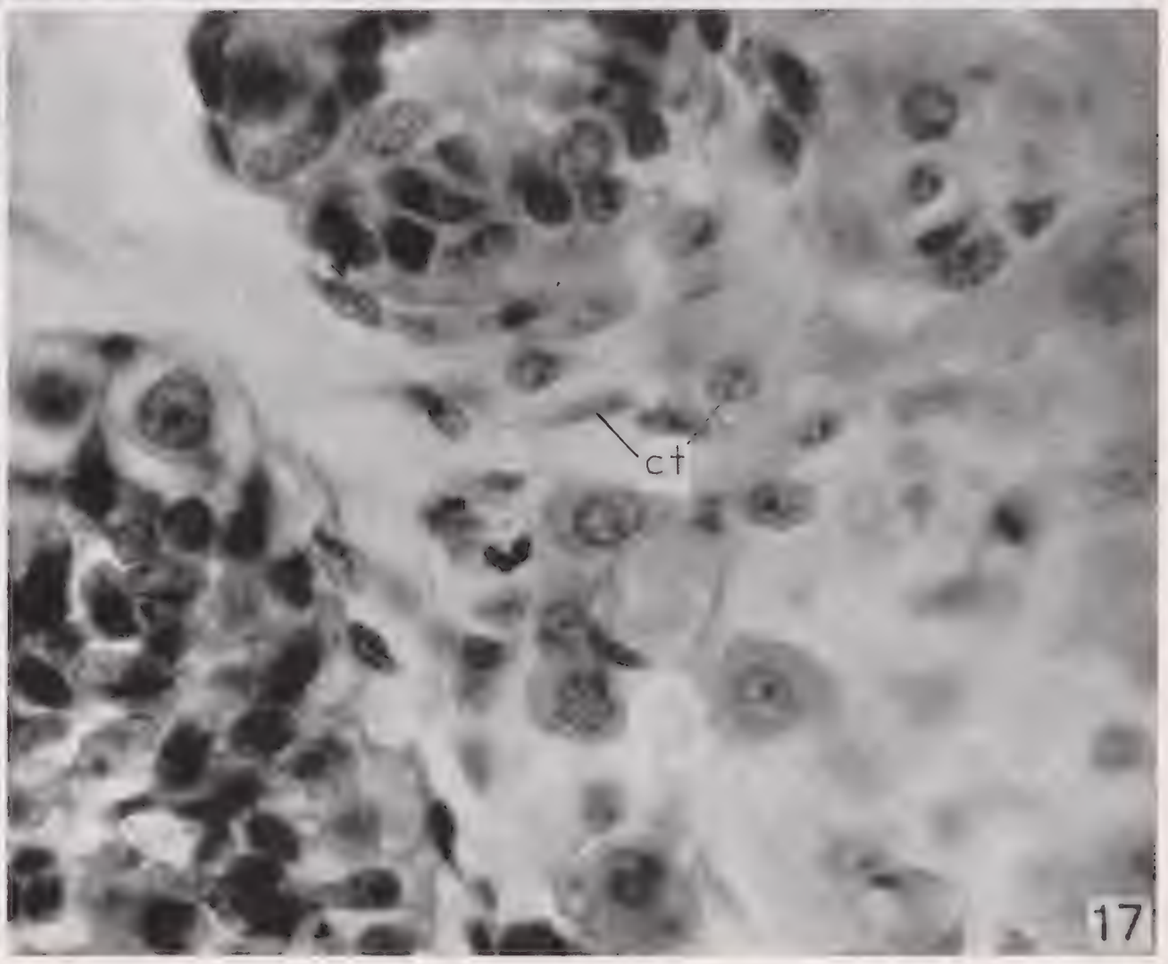


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FIG. 19. (Series 4304. C.-R. 25 mm.) Detail from figure 18, ×200. Note the absence of any cordlike structures in the cortex. The cordlike structure in the center of the ovary is the rete ovarii. The coelomic epithelium is ill defined.

FIG. 20. (Series 296. C.-R. 17 mm.) Section through the cortex of an ovary, ×800. The coelomic epithelium is irregular and continuous with the cellular area of the cortex. There is no well marked tunic. The cells of the cortex are of three kinds: (*a*) spindle-shaped mesenchymal cells, (*b*) large primitive sex cells, (*c*) intermediate pregranulosa cells. There is evidence of cords. The small cells of the genital cords are relatively much larger than in the male. Remains of cords are evident in the loose clusters of cells.

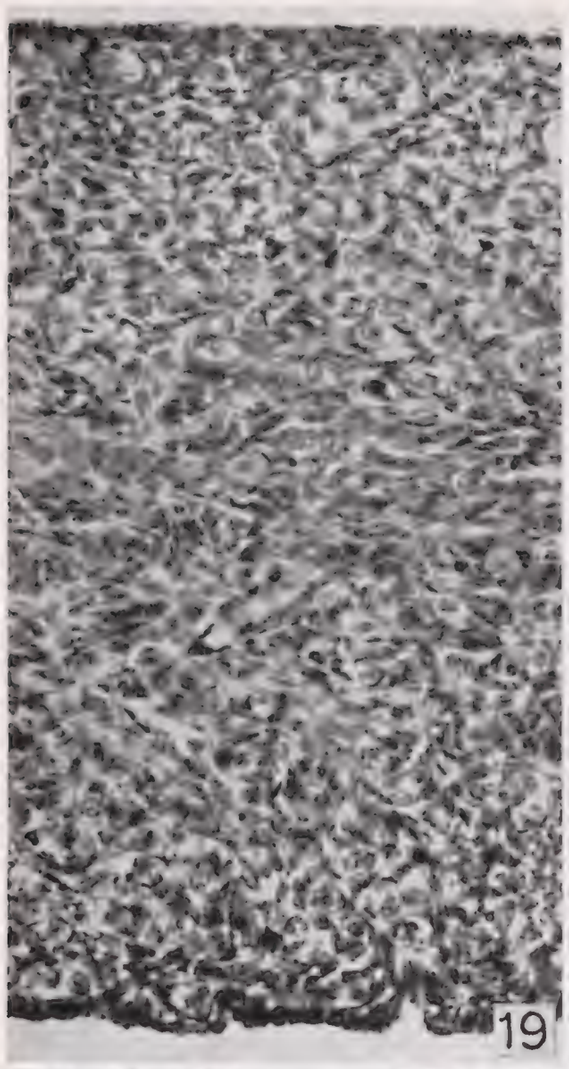
FIG. 21. (Series 296. C.-R. 17 mm.) Section through the medulla of the same ovary shown in figure 20, ×800. There is a cordlike arrangement of cells in the medulla, seemingly separated by spindle-shaped mesenchymal cells (*mes*) from the cellular cortex (*cor*). Sex cords are very much thickened in this region, and primitive sex cells (*psc*) are recognizable by their size.

FIG. 22. (Series 6573. C.-R. 31.5 mm.) Transverse section through an ovary, illustrating cortex and medulla, ×100. Mesenchyme tissue in the medulla surrounds the rete ovarii.

FIG. 23. (Series 6573. C.-R. 31.5 mm.) Detail from figure 22, ×200. The coelomic epithelium is highly active, with a mitotic figure present at *psc;* there are many large cells, some of which are undergoing division. Three types of cell occur in the cortex, as described for figure 20. The medulla contains the rete cords (*rc*) with hyperchromatic nuclei.

FIG. 24. (Series 1908. C.-R. 100 mm.) ×48. This specimen illustrates the effects of bad fixation on the structure of the ovary.

FIG. 25. (Series 12-10 A, Forbes' collection. C.-R. 109 mm.) ×200. This section shows the relatively undifferentiated character of the ovary at this late stage of development. The sparsely disposed mesenchymal cells (*mes*) outline and accentuate the massive sex cords.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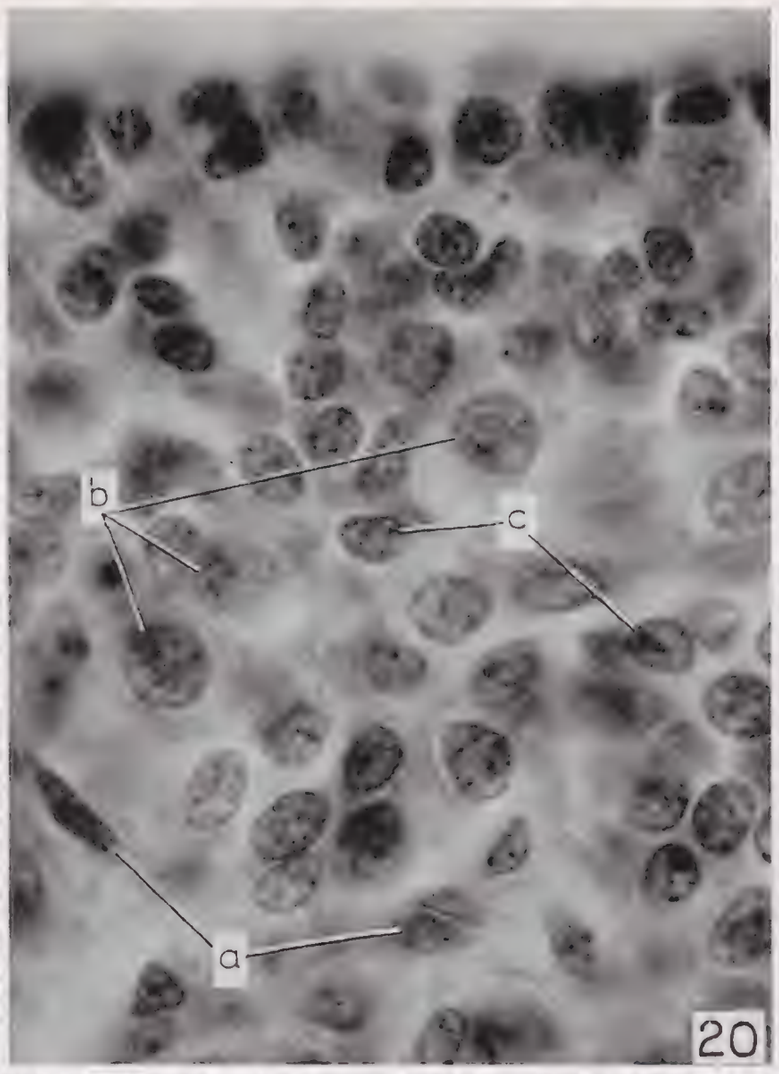

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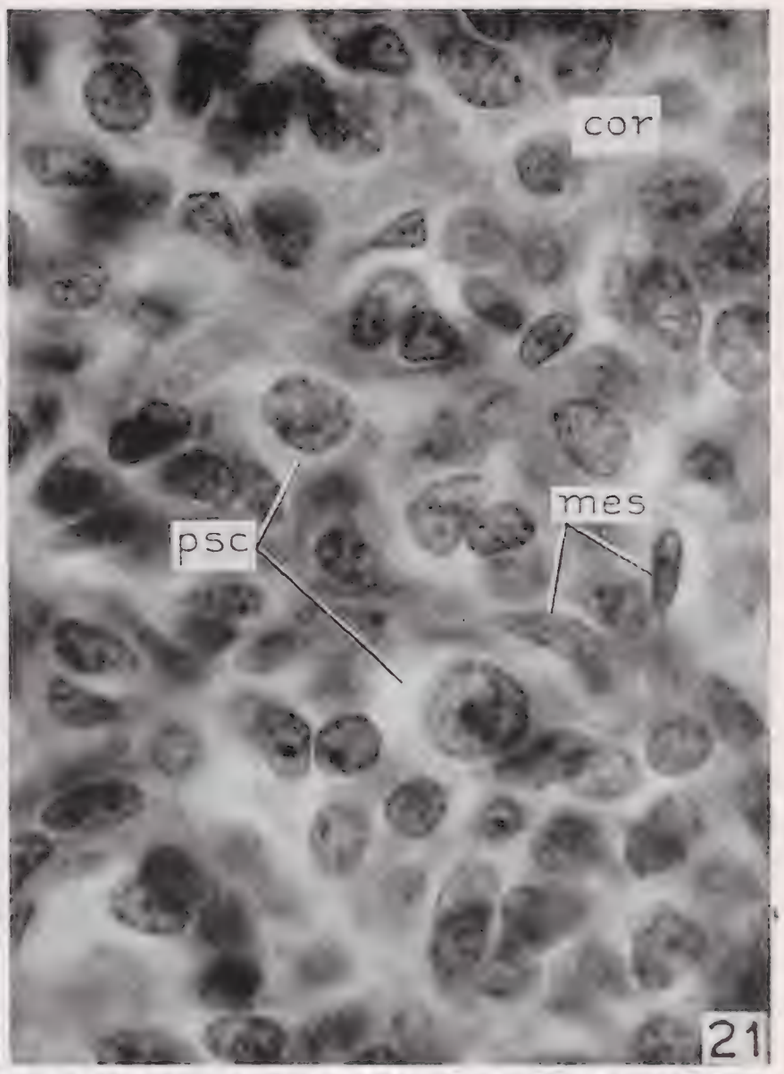

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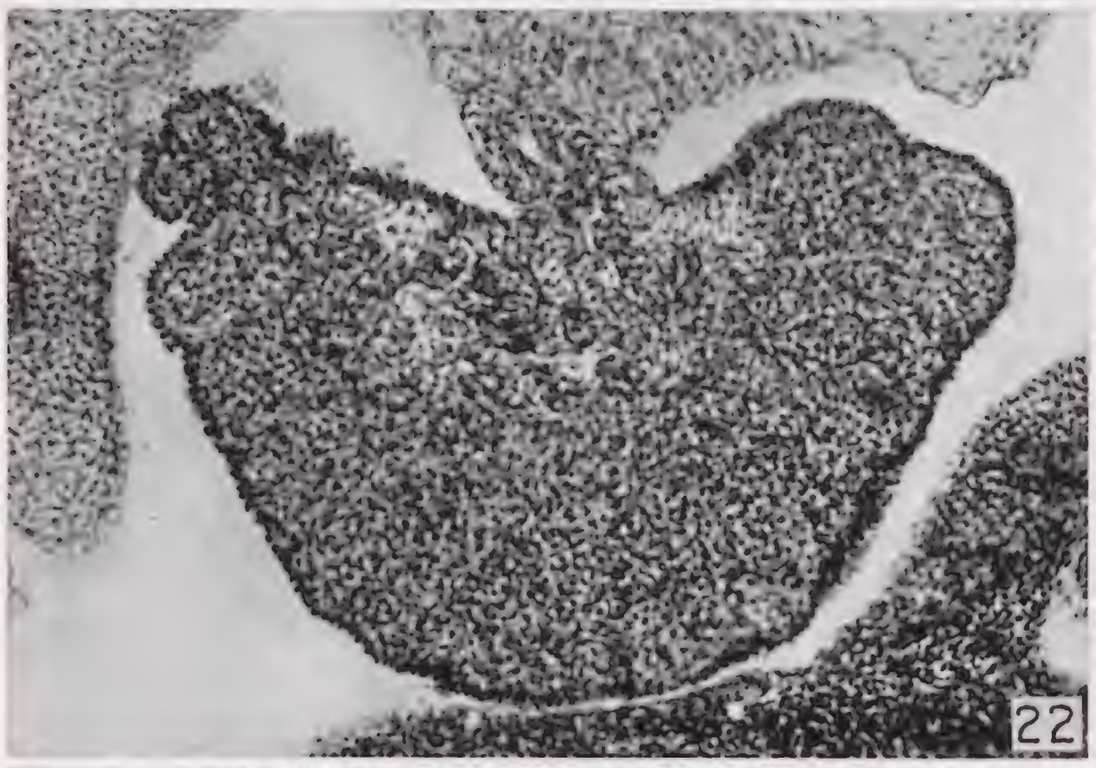
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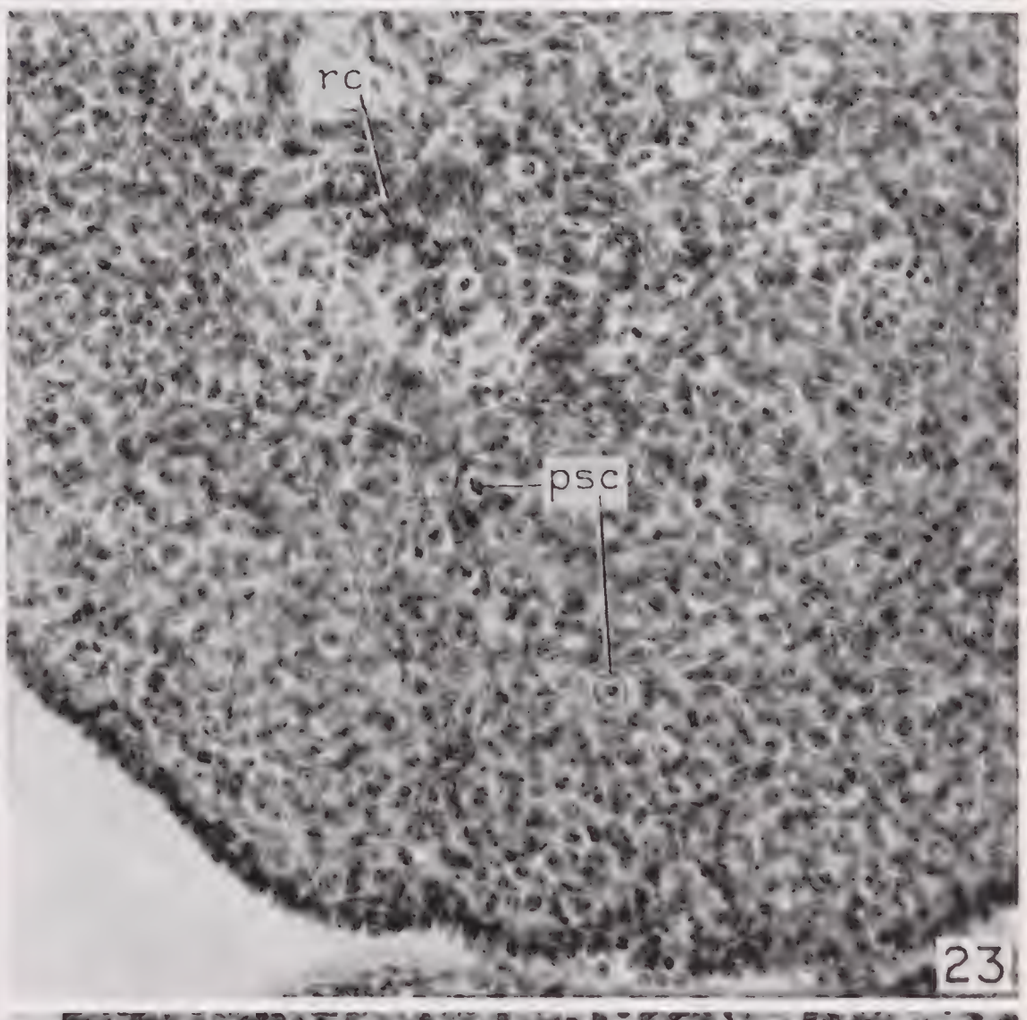

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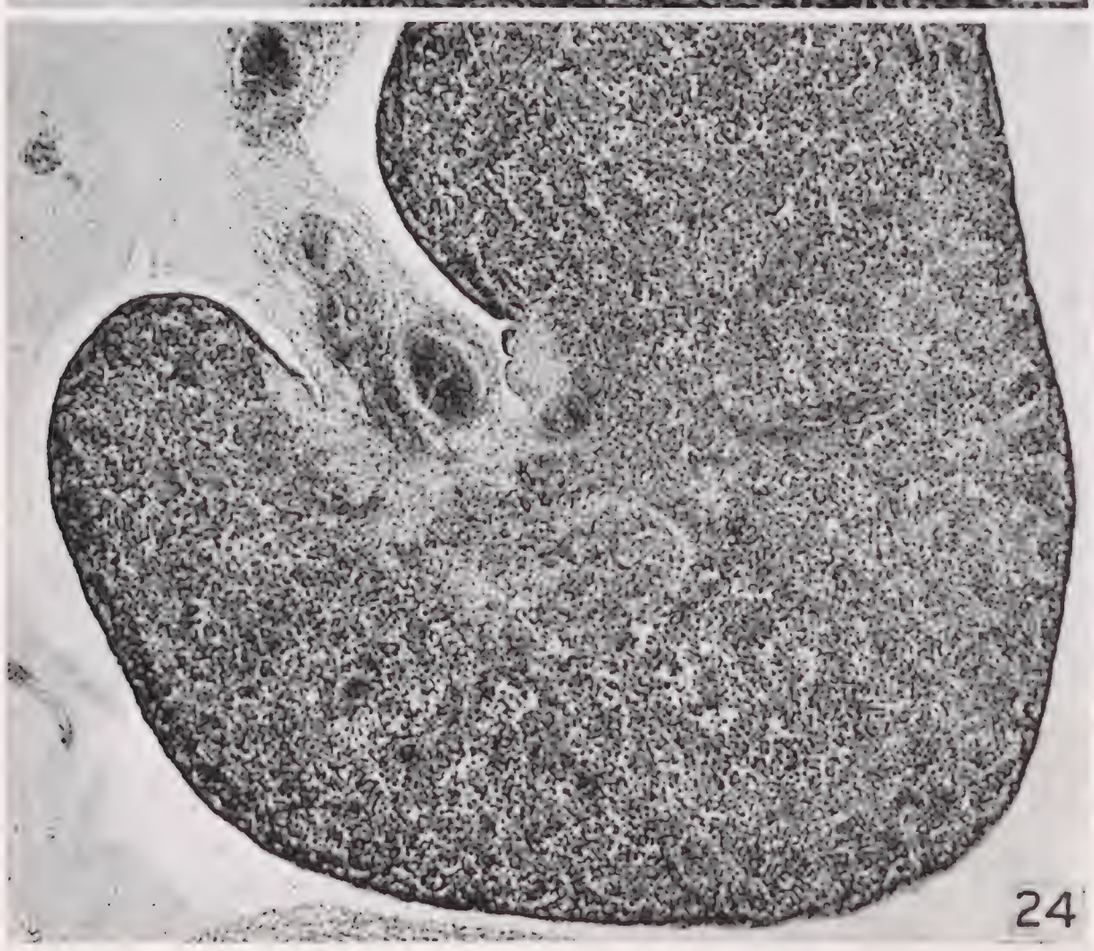
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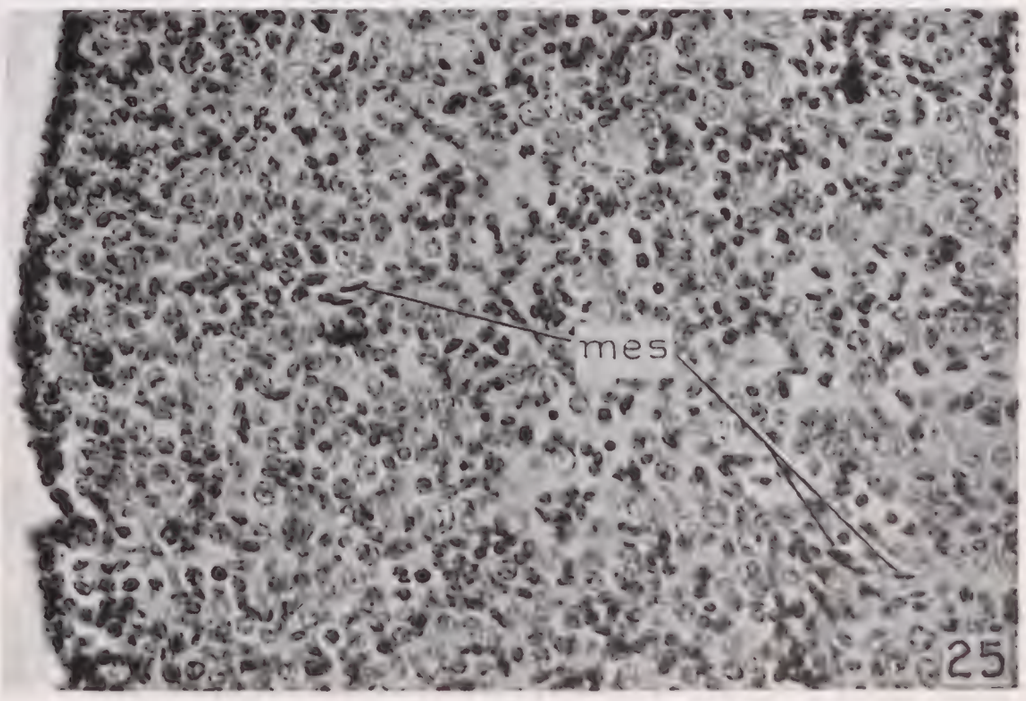

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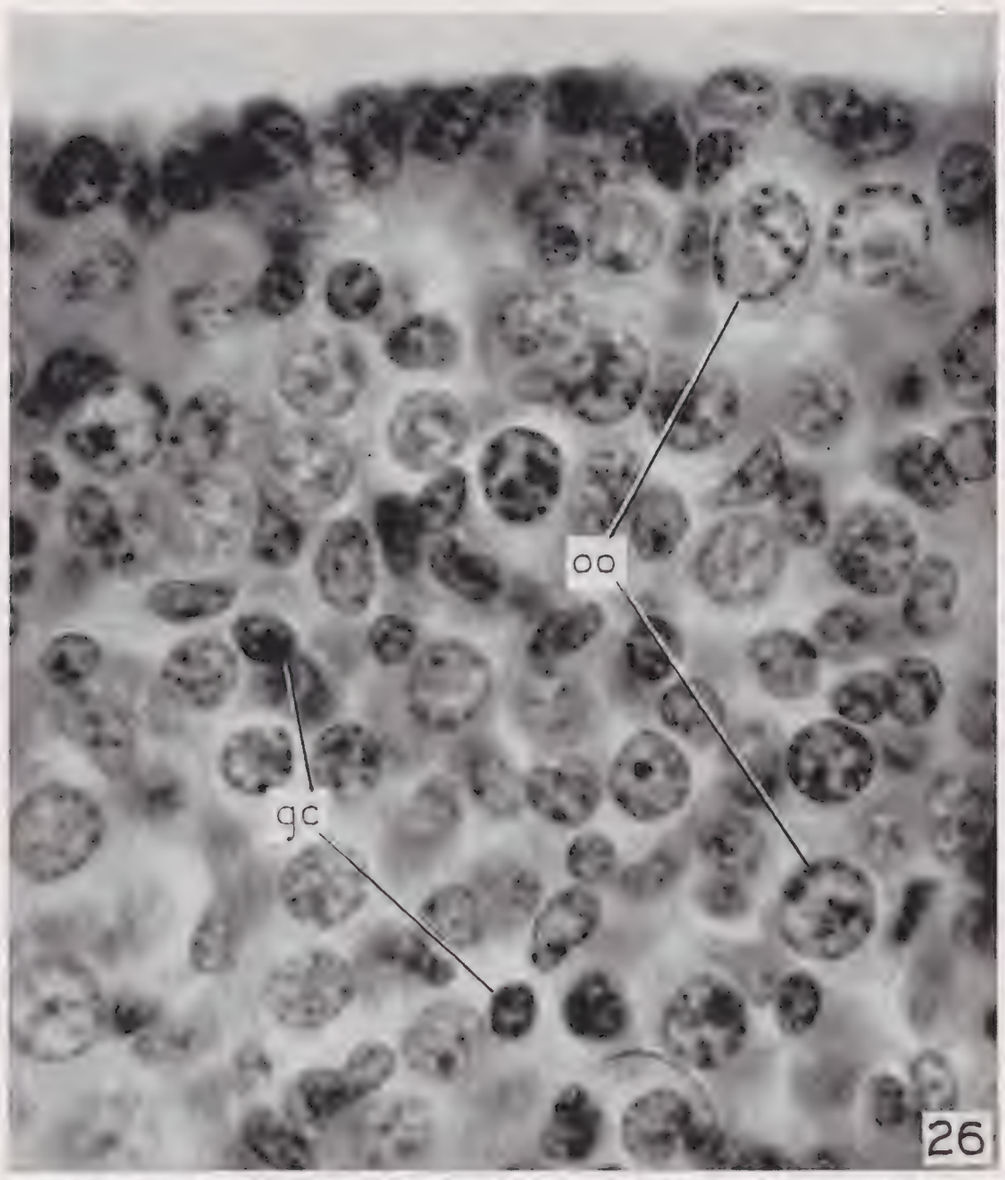
FIG. 26. (Series 12-10 A, Forbes' collection. C.-R. 109 mm.) Detail from figure 25, ×800. Cells of the germinal epithelium are still inseparable from those of the ovarian cortex. The cells with large round nuclei, some showing chromatic figures, are germ cells (*oo*). The small cells (*gc*) will form the granulosa cells of the follicle.

FIG. 27. (Series 12-11 A, Forbes' collection. C.-R. 190 mm.) ×200. The arrangement here shows broad cords of cells which simulate the "tubes of Pflüger." The period between 170 and 190 mm. C.-R.L. represents a period of growth during which important changes occur in the ovary. Fetal stroma appears in the medulla; sex cells enlarge to form oögonia and become encapsulated by granulosa cells. The original septa dividing the sex cords are now thickened and may be regarded as forming the lining of the tubes of Pflüger. Note especially the degeneration of the sex cells in advance of the invading fetal stroma. These cells (*doo*) are indicated by their hyperchromatic and irregular nuclei.

FIG. 28. (Series 7774. C.-R. 221 mm.) Cortex and part of medulla, ×200. There are two areas in the ovary: the medulla, containing abundant fetal stroma and greatly enlarged sex cells with their capsules of granulosa cells; and the cortical region, in which the sex cells are beginning to enlarge in advance of the invading stroma. Some of these sex cells have not yet become encapsulated. Degenerating oögonia (*doo*) are again indicated by pycnotic nuclei. The original "tubes of Pflüger" have become disrupted by the invading stroma. Compare with figure 27.

FIG. 29. (Series 7774. C.-R. 221 mm.) Medulla of same ovary as figure 28, ×200. There is great hypertrophy of fetal stroma, and a cord of tissue containing egg cells and granulosa cells which are much larger than the stroma cells. This is the remains of the medullary cord (*mc*). Degenerating egg cells (*doo*) are seen in the deepest part of the cortex. Egg cells in the cord (*mc*) are similar to occasional spermatogonia incorporated into the rete testis or straight tubules. Follicles are larger in the medulla than in the cortex. Compare with figure 28.

FIG. 30. (Series 12-30, Forbes' collection. C.-R. 272 mm.) ×200. The fetal stroma has invaded almost the whole thickness of the cortex. Follicles in the deeper part of the cortex are larger than in more superficial areas; and the granulosa cells of such follicles are much flatter than in those near the surface. Cords of follicular (granulosa) cells unrelated to ova (*cgc*) are shown. Such cords are frequently found extending for varying depths into the substance of the ovary; they occur in almost every ovary, to a greater or less extent, up to birth. These cords represent excess granulosa cells, existing probably as a result of heavy mortality among unencapsulated oögonia.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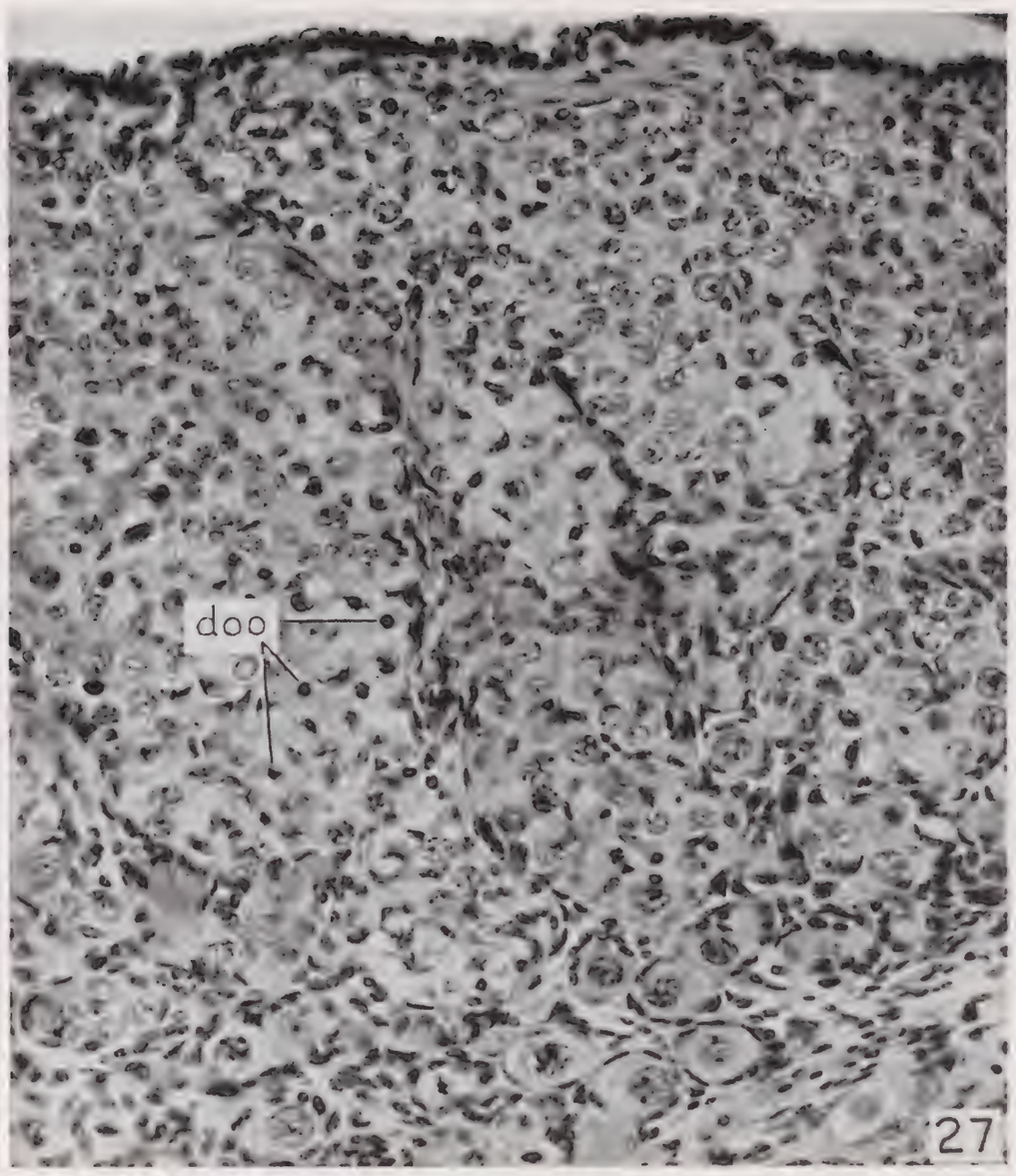
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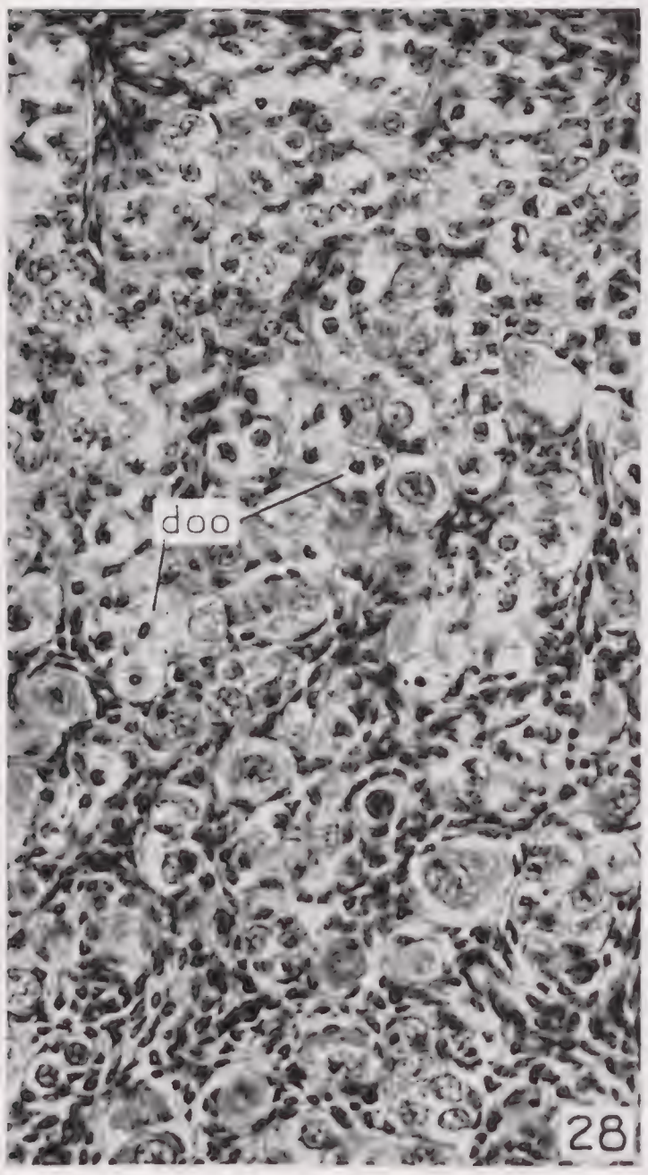
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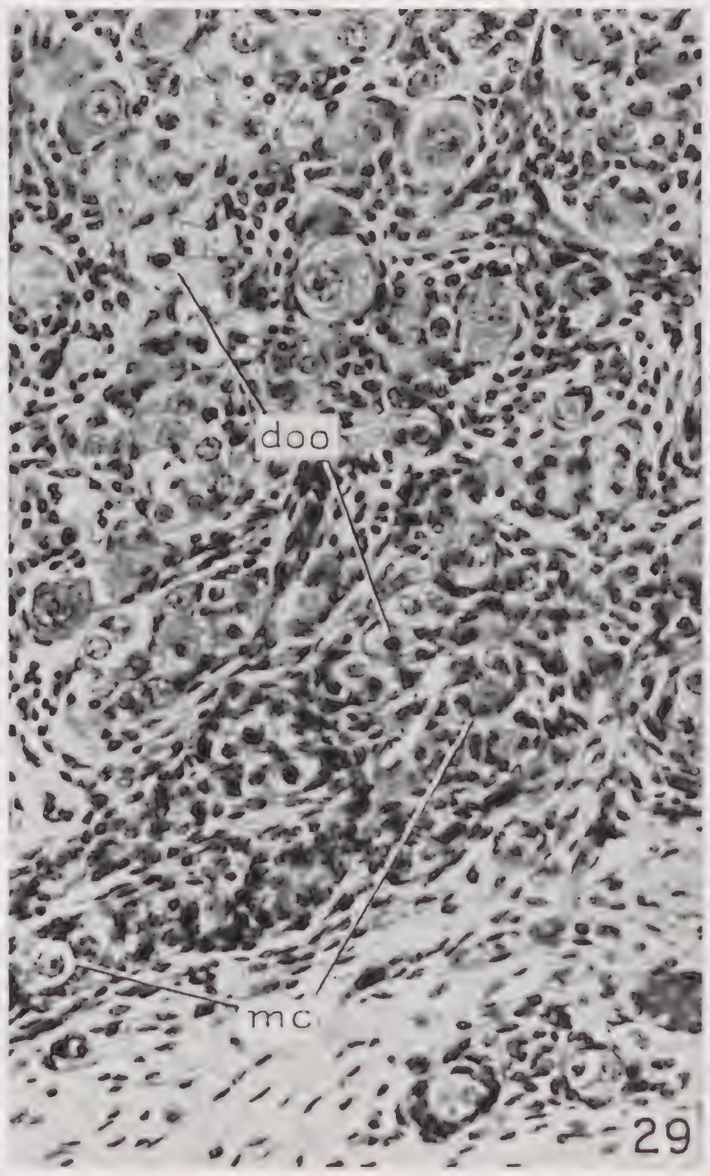
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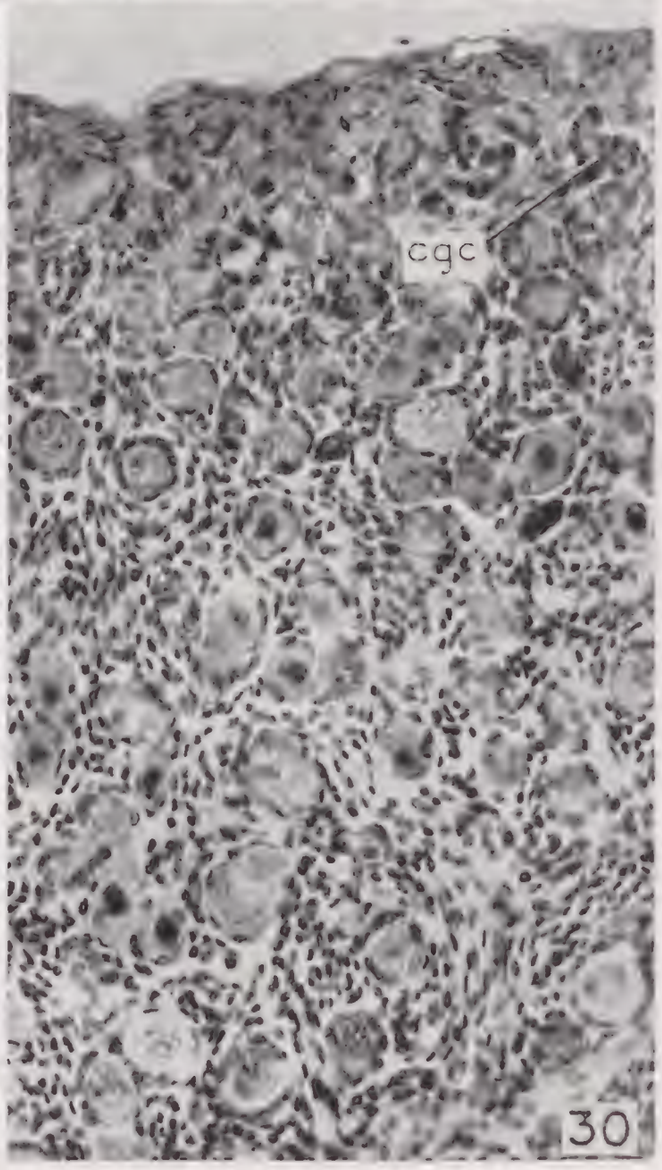
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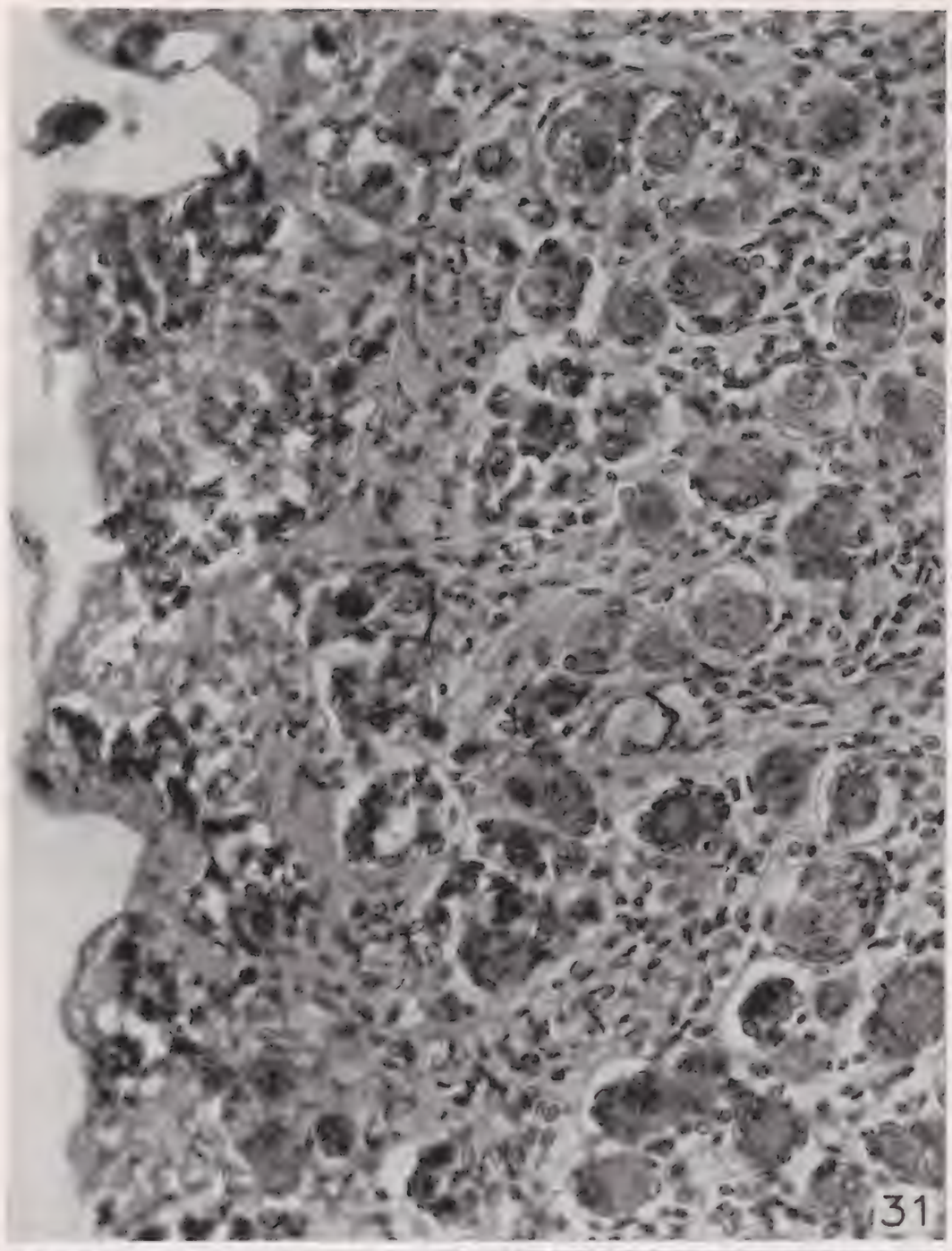
## Plate 6

Fig. 31. (Series 12-1, Forbes' collection. C.-R. 260 mm.) ×250. This section shows dense masses of enlarged granulosa cells in the superficial region of the cortex. These cells have invaded the clusters of egg cells while the latter were in the process of degenerating. Granulosa cells evidently were formed in excess of the amount required. These granulosa cells bear no resemblance to the cells of the fetal stroma. This is an exaggerated form of the process depicted in figure 30. The fetal stroma has not invaded the zone where the clusters of granulosa cells are found.

Fig. 32. (Series 4746. C.-R. 175 mm.) ×250. This section shows an intensification of the process represented in figure 31. Note the formation of cordlike structures, in continuity with the coelomic epithelium, which extend among degenerating clusters of egg cells. The absence of fetal stroma from this region is a characteristic feature.

Fig. 33. (Series 12-9 A, Forbes' collection. C.-R. 270 mm.) ×250. This picture is almost indistinguishable from some types of granulosa cell tumors. Note again the surface tubules and clusters of cells of different sizes. The overgrowth of fibrous tissue among these masses of cells is noteworthy. This is a continuation and intensification of the process indicated in figures 30, 31, and 32.

Fig. 34. (Series 12-9 A, Forbes' collection. C.-R. 270 mm.) ×250. Another region of the same ovary as that shown in figure 33. Note the great amount of fibrous tissue enclosing masses of egg cells and granulosa cells. There is almost complete absence of fetal stroma. Note the tubular character of the cell formations.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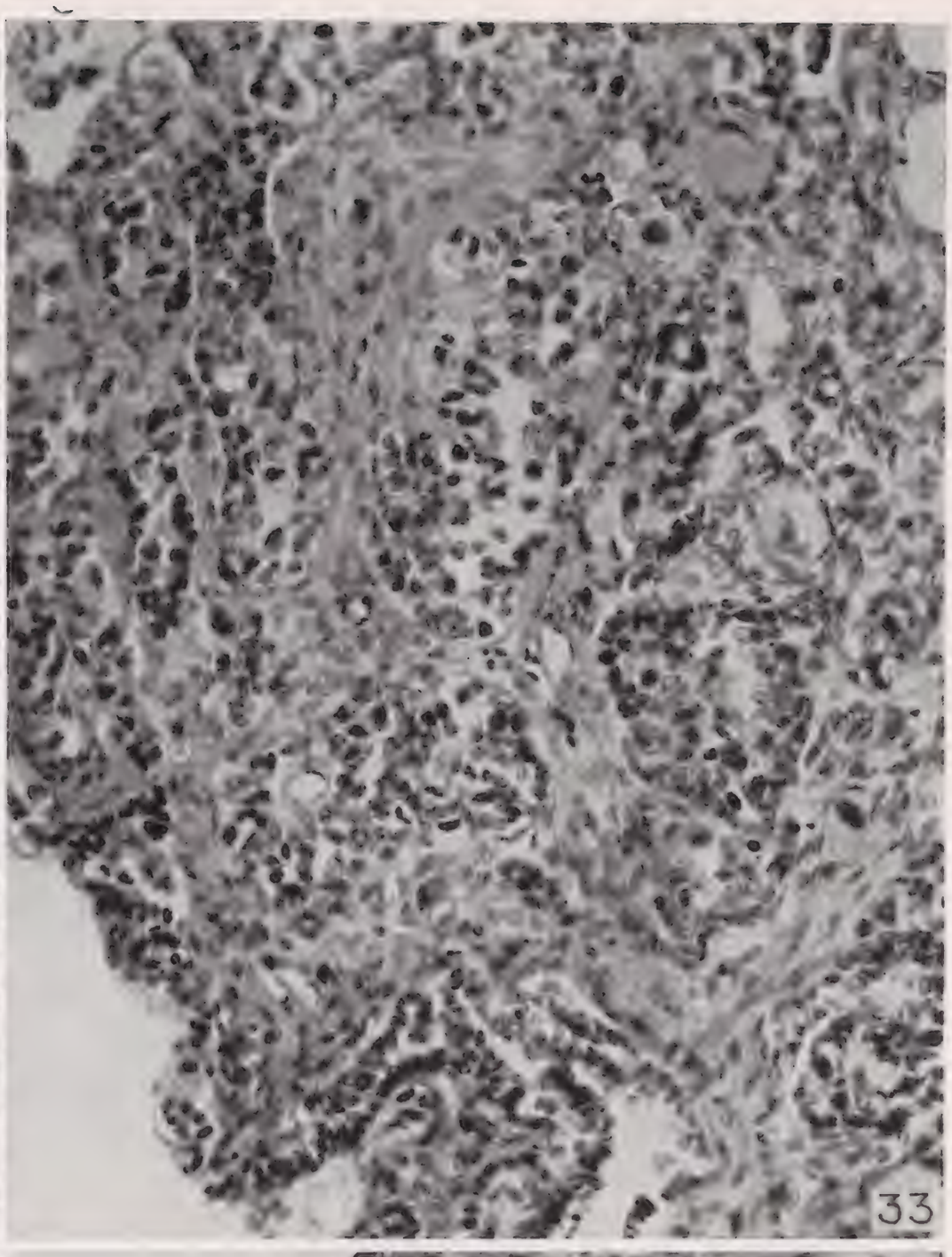
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CONTRIBUTIONS TO EMBRYOLOGY, NO. 211

# DEVELOPMENTAL HORIZONS IN HUMAN EMBRYOS

## Description of Age Groups XV, XVI, XVII, and XVIII, Being the Third Issue of a Survey of the Carnegie Collection

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Age group xv, three plates and seven text figures
Age group xvi, three plates and eight text figures
Age group xvii, three plates and eleven text figures
Age group xviii, three plates and ten text figures

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# DEVELOPMENTAL HORIZONS IN HUMAN EMBRYOS

## Description of Age Groups XV, XVI, XVII, and XVIII

## INTRODUCTION

### Syndromic Precision of Developmental Processes

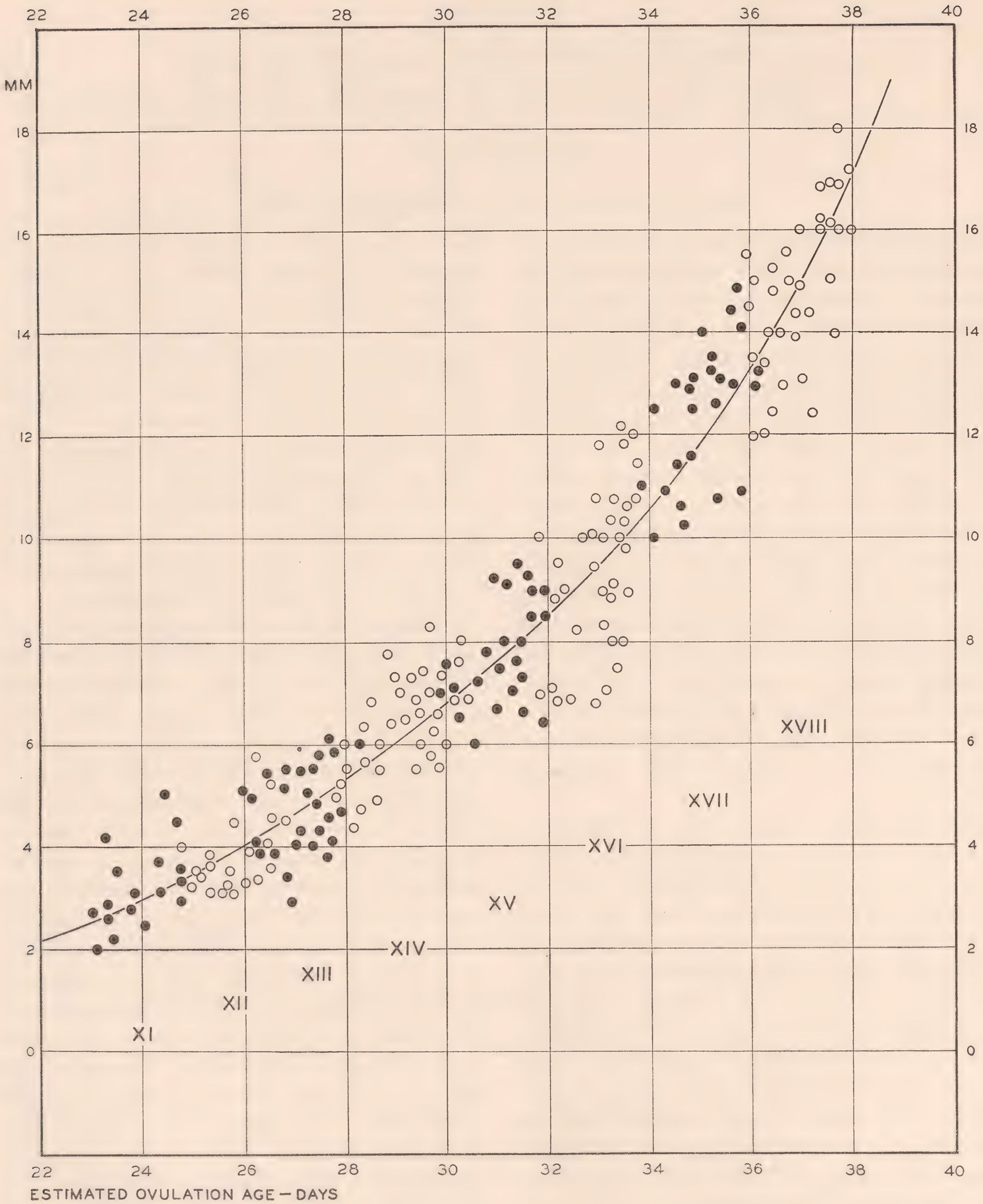
From the study of a large number of human specimens, such as is found in the Carnegie Embryological Collection, one is soon aware of the opportunity they provide for the preparation and description of an improved list of developmental stages. A new list, in itself, possesses for the embryologist a practical value that justifies its preparation. The present study, however, extends beyond the single purpose of a list of stages. It aims at a survey, as far as this is possible, of the successive developmental levels with reference to their functional potentialities, and particularly of the differences that mark the advance from one level to the next. In that sort of survey many interesting questions, bearing upon the structural adaptations of the organism to the increasing complexities of its functional requirements, are encountered. These specializations are the consequence of the obvious fact that the organism, at all levels, not only develops but also must keep alive and maintain itself during the process.

One of the questions which arise, as fundamental to the subject of embryonic adaptation, is that of the degree of correlation in the development of the different organs. Is this correlation a loose and variable one, or is it closely integrated and invariable? Do the embryological parts progress independently in unrelated order, or do they fit into a precise system, each unit of which comes into existence in an accurately timed sequence? If the latter is true, we should know, from the developmental status of one organ, the status of all the other organs in that particular specimen. From the kidney alone, or from the membranous labyrinth, or from the shaft of the humerus we should be able to reconstruct the whole embryo.

If our tissues and organs depend upon one another for their origin and form, the above is just what one should expect, and in consequence it would be found that our understanding of development must be based on the embryo as a whole and that we cannot restrict the field of analysis to individual organs any more than we can to individual cells. Furthermore, if development of the total organism is to be regarded as regulated by interactions between diverse cells and cell masses, and if there are inductor tissues and induced structures, a precise correlation in the main features of organogenesis would seem inevitable. Finally, if, for its normal development, each primordial cell mass is responsive to and dependent on influences from neighboring cell masses, it follows that an intricate and rigid schedule must be followed by the various embryonic units of the organism, and any marked departure from it is bound to open the way to abnormal and monstrous development.

In view of the above reasoning it has seemed important to compare, more carefully than has heretofore been done, the progress of development of the various organs with respect to one another and to determine how closely they conform to a rigid seniority schedule. This may be an appropriate place to record that this has now been done for the eight horizons (xi–xviii) thus far surveyed, and that throughout that range of development at least, it is found that a definite and invariable schedule of organ correlation does occur. At any one of the demarcated horizons, the growth and differentiation of the various body tissues and organs conform to a definite pattern, characteristic for that level of development. At each respective level one can recognize a syndrome of characters, and the presence of any one of these characters in a given embryo betokens the existence of the others. In the light of this syndromic nature of its organogenesis, one could compare the embryo to a form of intricate clockwork, in which each part is accurately geared, and it follows that these component parts have no basis or meaning beyond their relation to one another and to the embryo in its entirety.

In the following descriptions of the individual horizons, various features in development will be pointed out that are closely correlated and constitute syn-




A graphic plot of specimens in the Carnegie Embryological Collection which have been surveyed and assigned to eight separate developmental horizons, the oldest four of which (xv–xviii) are described in the present issue. The arbitrary horizons are indicated by Roman numerals, and each covers about two days of development. Embryos belonging to odd-numbered horizons are represented by black dots, and those of even-numbered horizons by white circles. This is done so that the group assignment of borderline specimens can be determined. The estimate of age was arrived at by matching the specimens with macaque embryos of known ovulation age.

dromes characteristic of their respective horizons. Attention has been especially given to those characters that can be clearly and easily recognized and that do not require special staining techniques, or elaborate reconstructions, for their identification. So far as possible, they are characters that can be recorded as present or not present. For the purposes of the author such lists became imperative, but it is hoped that they may also be of use to embryologists beyond the precincts of the Carnegie Laboratory. With these lists before one, it should be possible to survey the serial sections of any new embryo and in a brief time arrive at its proper horizon, and its ovulation age. It remains to be noted that if there were not actually a syndromic occurrence of discernible characters at successive levels, it would not have been possible to arrive at a subdivision of the ontogenetic history of the embryo into discrete developmental horizons, such as have been adopted in the present survey.

## Age Groups Covered in Present Issue

Two previous issues of these studies of the Carnegie Embryological Collection have appeared in volumes 30 and 31 of the Contributions to Embryology, embracing four developmental horizons (xi–xiv). In the present issue the succeeding four horizons (xv–xviii) are added. The whole period thus far covered and the overlapping of the groups is shown in the accompanying graph. Each embryo is entered separately; the odd-numbered horizons are represented by solid black dots and the even-numbered ones by white circles, so that their assignment can be distinguished where the groups blend. In nearly all cases the embryos are sectioned serially and their membership in the respective horizons is based on their inner structure in terms of selected definable characters. In the plotting of the individual specimens their ordinate level is determined by their greatest length in millimeters, whereas their abscissal position is determined by their developmental characters. The younger members of a horizon are spaced toward the left and the older members toward the right; such spacing, however, can be only approximately accurate. The age is an estimated one based on resemblance to macaque embryos of known ovulation age. During this period of development, comparison with macaque embryos appears to give more reliable age data than the clinical histories obtained from patients. As a whole, this method of plotting embryos on the basis of their inner structure gives us a better curve of growth than has hitherto been available; also it gives a comprehensive view of the number and dispersal of the embryos in the periods under consideration.

## HORIZON XV

### External Form, Size, and Age

Up to this time the central nervous system has played the principal role in determining the contours of the head and trunk of the embryo. Other details in its form are provided by the heart, the limb buds, and the condensed masses which are to form the mandibular and hyoid regions (the pharyngeal bars). As we have seen in the preceding age group, the embryo resulting from these influences is a bilaterally flattened object, having a curved or partially spiral axis. In the embryos which are now before us, the relative width of the trunk region (pl. 1, xv) has become greater owing to growth of the spinal ganglia, the somites, and the mesenchymal tissues associated with them, all of which add to the bulk of the trunk. Thus when viewed from the back, embryos of age group xv appear wider from side to side than those of age group xiv. This increase in relative width of the trunk becomes still greater in the succeeding age groups. The result is that the greatest transverse diameter, which originally is in the dorsoventral axis as in a fish, eventually coincides with the side-to-side width of the embryo. In the four age groups xv to xviii this increase in relative width of the trunk is rapid enough to serve as one of the criteria for determining the developmental horizon in which a given embryo belongs. Allowance must always be made, however, for the fact that the occipitocervical region of the trunk is more advanced and is consequently wider than the lumbosacral because of the cephalocaudal gradient in growth. New factors greatly complicate the picture in older age groups, where special modifications occur in the relative diameters of the trunk in its different regions and where its width varies correspondingly.

The range of variation in the external form of embryos belonging in this age group is illustrated in plates 1 and 2, xv. The particular variations in each specimen are mentioned in the descriptions of the plates. Those details will not be repeated here. It will be sufficient to call attention to five characteristics that are present in all of them: (1) The lens vesicles have closed, and on each side the pores by which the vesicles had communicated with the surface have disappeared. (2) The olfactory placodes, owing to the relatively greater growth of the surrounding tissues, in this age group begin to recede from the surface, acquiring the form of large oval depressions. In the younger members a low ridge marks the frontal and lateral borders of the depression. In the older members of the group this ridge has become a slightly overhanging lip. It is this rapidly growing ridge that will subsequently form the nostril on its side of the head. (3) Before this period the hyoid bar has already been differentiated into a dorsal and a ventral segment, but now its ventral segment acquires a subsegment, or most ventral segment, which is the primordium of the antitragus. (4) The active transformations occurring in the arm buds, in the group under consideration, contribute to the precision with which embryos can be identified as belonging, or not belonging, in this age group. In horizon xiv we could speak of an elongating arm bud. In the present age group, as shown in plates 1 and 2, xv, one can recognize the developmental steps by which the arm bud becomes regionally subdivided into a distal hand segment and a proximal arm and shoulder region. The less precocious leg bud exhibits a beginning differentiation into a rounded rostral half and a more tapering caudal half. It is the tip of the latter that will form the foot. (5) As in younger embryos, in all the members of this age group the somites and underlying spinal ganglia produce characteristic elevations which can be seen, sufficiently well for fairly accurate counting, throughout the length of the cord, from the occipital region to the coccygeal levels. In transparent specimens they appear as white opaque condensations. In subsequent horizons the ganglia and somites will become covered over with mesenchymal tissues so that they can no longer be seen from the surface. This occurs first in the occipitospinal region, whence it spreads caudalward.

In considering the size of embryos one must make allowance for the type of fixation and any other treatment that takes place before the embryos are measured. Those details are not always accurately entered in the records, and the appropriate allowance may not be easily determined. One is not warranted, there-

fore, in placing emphasis on small differences in such measurements. Ordinarily embryos are measured subsequent to fixation and after they have been transferred to the weaker alcohols. At least, that procedure was followed with most of the embryos in the group we are studying. It will be seen in the "List of embryos" (p. 151) that the greatest length of each of the 25 specimens is entered under the heading "Size." If we omit 3 specimens which are either exceptionally small or large, there remain 22 which may be regarded as representative of the age group. Among these are 4 large ones having a length of 9 mm. or slightly over, whereas the remaining 18 specimens (82 per cent) range from 6.5 mm. to 8.5 mm. This range then may be taken as the expected length for the group. In general, the younger members of the group are found among the smaller specimens and the older members among the larger ones. If there were no variability in the technique the correlation between size and age would doubtless be closer, but even so one would not expect it to be precise. Of the 18 specimens constituting the 6.5- to 8.5-mm. range, 9 are 7.0 to 7.8 mm. long, that is, half have this intermediate length. Stating it in a simple way for the group as a whole, and with the exceptions above noted, it may be said that it is composed of 7- and 8-mm. embryos.

If precision is not to be had in embryo length, it is even less obtainable in the size of the chorion. The latter has usually been opened and is obtained more or less collapsed, and often one cannot be sure that the whole chorionic sac is present. The diameters of the chorion, however, were recorded for 16 of the 25 specimens and are entered for their gross value in our list of embryos, in the same column with the size of the embryo. In most of the specimens it will be seen that the greatest diameter of the chorion falls in the thirties, as was true of the preceding age group. One chorion alone had a greatest diameter reaching the forties (41 mm.). The embryo in that case was one of the older members of the group.

In previous horizons the estimate of age of the embryo was based on comparison with macaque embryos having a known ovulation age. Repeating that procedure here, the embryos of this group appear to fall between macaque embryo no. C 499 (8.0 mm., 31 days) and embryo no. C 482 (8.5 mm., 32 days).[1] In the former the lens vesicle still opens to the surface by a small pore, whereas in the latter it is closed and detached from the overlying epithelium as required for the present age group. Therefore no. C 482 is taken as a better match, and the estimate of the ovulation age for most of the younger members of this group is placed at 31 days, and for the older ones at 32 days.

We may now briefly summarize the distinguishing features that characterize the external form of the members of this age group. The lens vesicles are no longer open and the closure pores have disappeared. The olfactory placodes are beginning to submerge as oval depressions whose ridgelike borders, among the older members, take the form of an overhanging lip. The ventral segment of the hyoid bar shows the first indication of the primordium of the antitragus. The arm buds have become regionally subdivided into a distal hand segment and a proximal segment that will form the arm and shoulder. The leg buds exhibit beginning evidence of regional differentiation. As for size, the group is composed for the most part of 7- and 8-mm. embryos, and they are accompanied by a chorion whose greatest diameter, with a few exceptions, ranges between 30 and 40 mm. Matching these various characters with their counterparts in macaque embryos of known ovulation age, we arrive at an age estimate of between 31 and 32 days for the group.

## Olfactory Pit Compared with the Lens Vesicle

In the earlier levels of development we have seen how the neural tube by its very massiveness is the prime determiner, at that time, of the form of the embryo. In the present horizon, supplementary factors are entering as form determiners. Two notable ones are the neuroectodermal ear vesicle and olfactory plate, which appear to induce changes in the adjacent mesenchyme. The proliferation of the latter around the ear vesicle creates the primordium of the otic capsule, which, in addition to meeting exactly the requirements of the membranous labyrinth, is destined to form an early and prominent part of the chondrocranium. The mesenchymal specialization associated

[1] C. H. Heuser and G. L. Streeter, Development of the macaque embryo, Carnegie Inst. Wash. Pub. 525, Contrib. to Embryol., vol. 29, pp. 15–55, see pls. 31, 33, 1941.

with the olfactory plate plays, as we shall see, an important part in the formation of the nose and associated respiratory passages. In earlier horizons it has been pointed out that that part of the evaginated brain wall which constitutes the eye cup induces a specialization of the overlying skin ectoderm to form the lens vesicle. To this hierarchy of neural organizers we should perhaps add various peripheral neural elements. It has been shown by Bartelmez and Evans [2] that in the early somite stages the cranial nerve commitiated in the primordium of the lower jaw in man is the mandibular nerve. Later we shall have occasion to return to this neural priority and to observe how closely the primary trunks of the lumbar and sacral plexuses are related to the organization of the leg bud. In view of these circumstances we do well to bear in mind, in our study of organogenesis, the high rank among the many regulating influences that is taken by the neural ectoderm and its derivatives in the matter of dominance over other embryonic tissues.

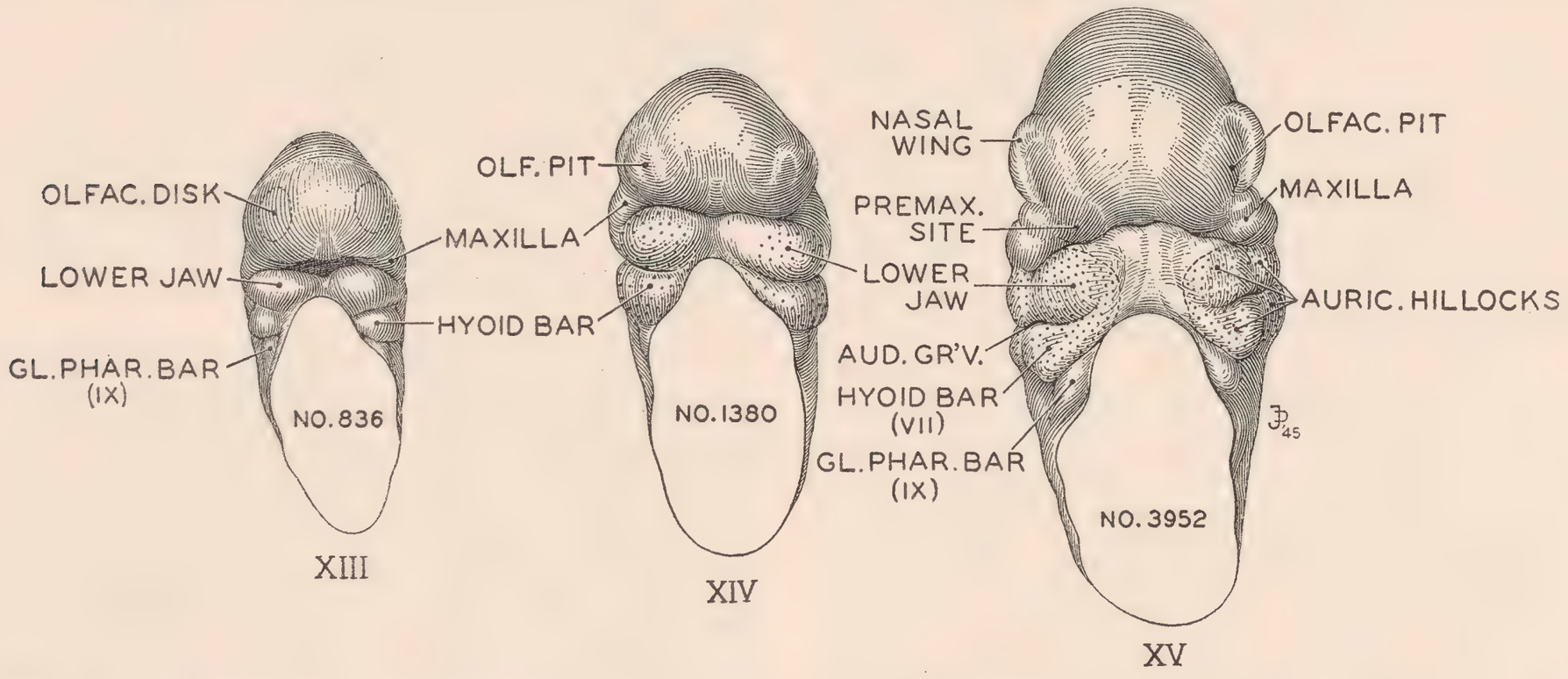



FIG. 1, XV. These three embryos, representing horizons xiii to xv, illustrate the transformation of the two flat olfactory disks or placodes into pits from which will be derived the nasal passages. This figure is to be compared with figure 2, xvi, figure 1, xvii, and figure 1, xviii. Together they form a series, at the same magnification, illustrating the morphogenesis of the face. The prominent lateral border of the olfactory pit in horizon xv (embryo no. 3952) is the primordium of the nasal wing and has usually been designated as the lateral nasal process. The median border of the pit is less prominent, but out of deference to its associate is commonly spoken of as the median nasal process. It is to be noted, however, that if the premaxillary center is not included with it, it plays but the small role of becoming the median rim of the nostril. In the three age groups illustrated in this figure, the mandibular bars unite in a definite lower jaw. The upper jaw is less precocious and is represented only by the widely separate right and left maxillary centers. One can see, however, where the premaxillary centers will form. The nerve trunks of the mandibular division of the trigeminal nerve appear to the casual glance more advanced than those of the maxillary division, and thus are associated with the precocity of the lower jaw. Drawings made by J. F. Didusch from reconstructions made by O. O. Heard. ×12.5.

plexes are more or less precursors of the condensation of the mesenchymal tissues which, in the horizon under consideration, can be recognized as the primordia of the maxillary, mandibular, and hyoidal masses. That is to say, among the first morphological events in the formation of a pharyngeal bar, aside from the activity of the overlying ectoderm, appears to be the occupation of the region by a proper branchial nerve. In fact, it was long ago observed by Fawcett [3] that the first structure to become differen-

Certainly the olfactory neural ectoderm takes a conspicuous part in the development of the face. In horizon xv the lower jaw is clearly recognizable but one cannot yet speak of an upper jaw. Aside from the small rudiment that will form the maxilla, there is nothing to represent the upper lip or nose, except the two olfactory pits. This region is shown for three successive age groups in figure 1, xv. In the youngest (xiii) the olfactory placodes consist merely of areas of thickened neural ectoderm. They scarcely influence the surface form and must be verified by section. This is also true of the near-by area of skin ectoderm that is to form the lens vesicle. The only notable difference between the two at this time is the close and

[2] G. W. Bartelmez and H. M. Evans, Development of the human embryo during the period of somite formation, Carnegie Inst. Wash. Pub. 362, Contrib. to Embryol., vol. 17, pp. 1–67, 1926.

[3] E. Fawcett, Ossification of the lower jaw of man, Jour. Anat. and Physiol., vol. 39, pp. 494–495, 1904–1905.

definite postural relation of the lens plate to the evaginated eye cup, to which Lewis [4] has shown its induction is to be attributed. In contrast, the olfactory plate overlies an area of seemingly indifferent mesenchyme, and is quite separated from the brain wall. Evidently it has within itself the dominating factors that are responsible for the changes which now rapidly follow.

Advancing to the next age group (xiv), the olfactory plate has undergone active cell proliferation with the result that it is larger and thicker. It has also acquired a shallow central depression, and its margins are elevated, forming a low enclosing ridge. The plate is still separated from the brain wall by a moderately wide area of indifferent mesenchyme in which many young capillaries are forming. Directly in contact with the plate, however, the mesenchyme exhibits a narrow zone of condensation. This localized proliferation appears to be induced by the olfactory plate. It is also quite possible that the vascularized mesenchyme on its part is influencing the overlying olfactory epithelium.

The lens vesicle has now become quite a different thing from the olfactory plate. It is smaller; it is cup-shaped; it exhibits the phenomenon of extrusion of unwanted cells; it does not induce proliferation of the neighboring mesenchyme, and instead lies in close adaptation to the concavity of the eye cup. Thus skin ectoderm in the case of the lens vesicle is being transformed into a specialized structure through the influence of the eye cup. In contrast, the olfactory plate consists of an ectoderm which from the outset contains neural elements. Together with the otic vesicle it is to be classed as a neural island or placode interset in the skin ectoderm. Both of them are to some extent capable of self-differentiation and of regulating the adjacent tissues.

Coming to age group xv, it is found that in consequence of active proliferation of the cells of the olfactory plate and its increase in surface area, a definite pit or invagination is produced by the folding over of its edges, particularly on its dorsolateral margin. What was a low ridge in age group xiv has now become a lip which in the older members of the group partly covers the floor of the pit. This lip is known as the lateral nasal process and will form the nasal wing on that side of the nose. The two nostrils are relatively wide apart at the outset, and we shall find that they become even more so in the next age group (xvi).

The lower jaw forms earlier than the upper jaw, perhaps reflecting the difference in composition (portio minor) of the mandibular division of the trigeminal nerve, as compared with that of the other two divisions. In figure 1, xv, one can follow the steps in the origin of the lower jaw. In addition to the median groove, marking the line of symphysis of the two halves, there are lateral and transverse grooves indicating multiple centers of the jaw. The lateral and caudal surfaces of the mandibular bar contribute to the formation of the external ear, shown by stippling in the figure. The lower jaw at this time clearly marks the lower border of the entrance to the stomodeum, but no such boundary marks its upper border. The only obvious representatives of the upper face are the olfactory pits. The primordia of the right and left maxillary masses are present, but these show little evidence, as yet, of the part they are to play in the formation of an upper jaw.

## Heart

In the matter of adjustment to new requirements, as the organism advances from one developmental level to the next, the vascular system is one of the most adaptable of the various structures of the developing embryo. In tracing, in the embryo, the venous drainage of the enlarging brain, the author [5] found that the venous pattern underwent a continuous series of modifications, and it became evident that many of the constituent channels of a blood pattern have ephemeral importance. On the other hand, some of them, owing to the circumstances of the environment, persist as adult vessels with only minor modifications. Whether a vessel is to be ephemeral or permanent is apparently regulated by the service it renders. Also, how ephemeral or how permanent it is, or what form it finally takes, likewise seems to be regulated more by the environmental requirements than by any particular quality of the vessel itself.

Under these circumstances it follows that an uninterrupted series of vascular transformations is taking

[4] W. H. Lewis, On the origin and differentiation of the lens, Amer. Jour. Anat., vol. 6, pp. 473–509, 1907.

[5] G. L. Streeter, The developmental alterations in the vascular system of the brain of the human embryo, Carnegie Inst. Wash. Pub. 271, Contrib. to Embryol., vol. 8, pp. 5–38, 1918.

place throughout the period we are considering, and such adaptations increase in number and complexity as the organs become larger and more specialized. To follow in detail these successive vascular patterns demands special material and techniques which render them unsuited for defining developmental horizons. They have been well described in several papers that have appeared in the Contributions to Embryology, Carnegie Institution of Washington, to which the interested reader is referred.[6] During this same time, however, the heart itself undergoes marked alterations, changing it from a relatively simple bulbous pump to a two-current pump with valves. It will be seen that these changes are sufficiently marked to be detected between successive horizons in ordinary serial sections. Thus the structure of the heart can be included with advantage in listing the syndromic features that characterize our developmental horizons.

In younger age groups (compare fig. 4, xi; fig. 4, xii; fig. 5, xii; fig. 2, xiii; and fig. 3, xiii) we have traced the origin of the venous end of the heart, that is, the sinus venosus and atria, from specialized parts of the vitelline plexus which form a vascular saddle astride the neck of the yolk sac. From the very outset the arterial part of the primordial heart, the part from which the ventricles are derived, is clearly demarcated from the venous part coming from the vitelline plexus. This is shown in figure 5, xii (stage of horizon x). The arterial part of the heart is further

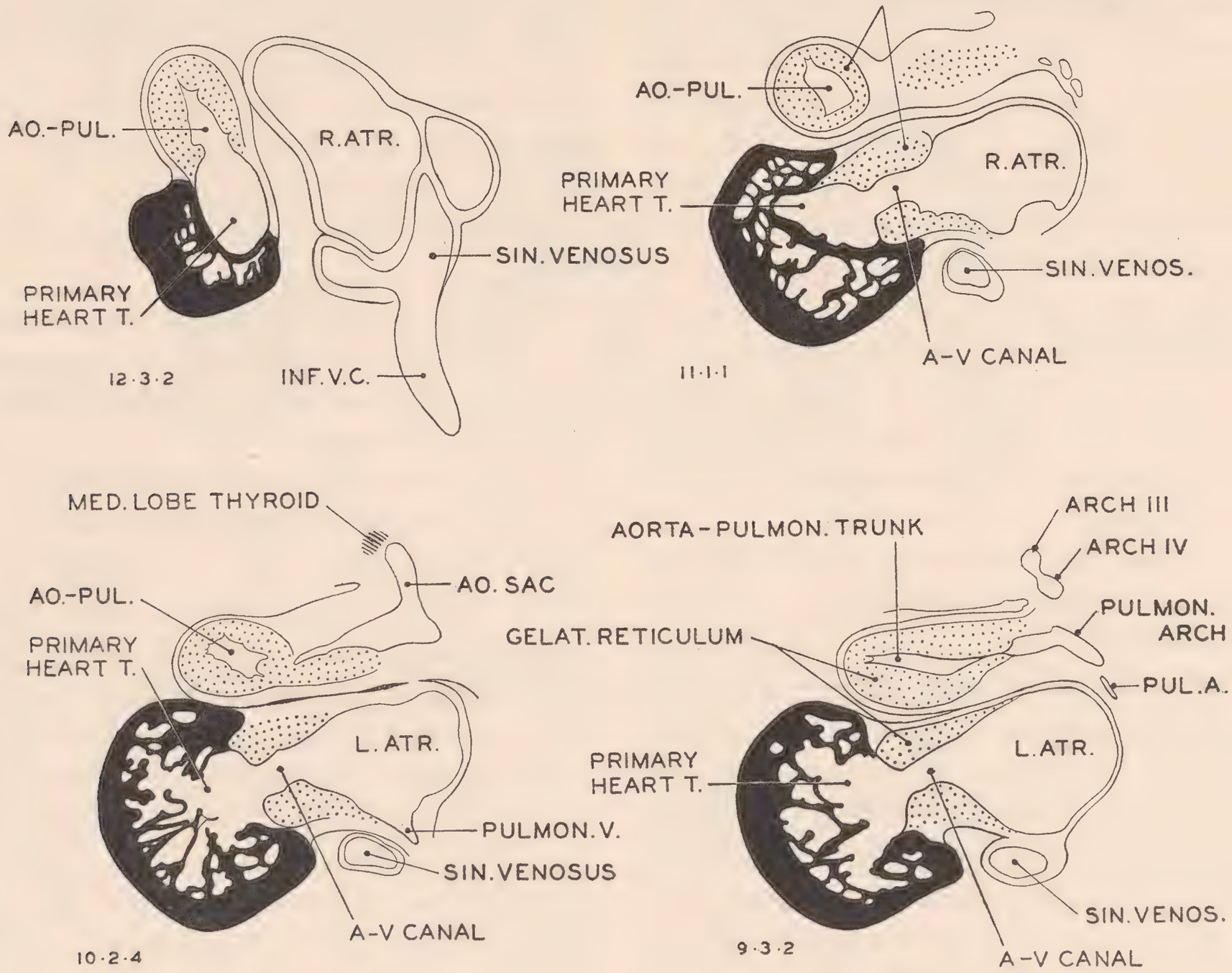



FIG. 2, XV. Selected sagittal sections through heart of embryo no. 6504, section number indicated under each section. This figure illustrates the fact that the heart is composed of three tissue types: the venous type of the atria; the gelatinoreticular type of the primary heart tube; and the highly specialized muscular ventricles, opening out of the primary heart tube. ×30.

[6] The following papers in the Contributions to Embryology are representative in covering the vascular system in different regions and at different developmental levels: F. R. Sabin, vol. 6, pp. 61–124, 1917; C. E. Buell, Jr., vol. 14, pp. 11–26, 1922; E. D. Congdon, *ibid.*, pp. 47–110; E. B. Finley, *ibid.*, pp. 155–162; H. H. Woollard, *ibid.*, pp. 139–154; C. H. Heuser, vol. 15, pp. 121–139, 1923.

illustrated in the accompanying figure 2, xv. Comparison of the four levels of development shown in the latter figure reveals that throughout that period one can speak of a primary heart tube that starts abruptly at the atrioventricular junction and curves upward to terminate in the aortic distributing sac. In contradistinction to the venous part of the heart and blood vessels in general, the primary heart tube is structurally distinguished by an enclosing sleeve or layer of cardiac jelly which fills the myoendocardial space throughout its whole length. At first this layer is almost a clear jelly, as described by Davis.[7] It appears to act as a closure cushion that facilitates the emptying of the tube and at the same time checks regurgitation backward from the aortic sac, biding the time when specialized cardiac valves will become established. In arriving at the more advanced horizons the layer of cardiac jelly acquires more cells, and one then speaks of it as a gelatinous reticulum. In more condensed form we shall see this converted into the semilunar valves, and it finally disappears in the formation of the tricuspid and mitral valves and the membranous interventricular septum.

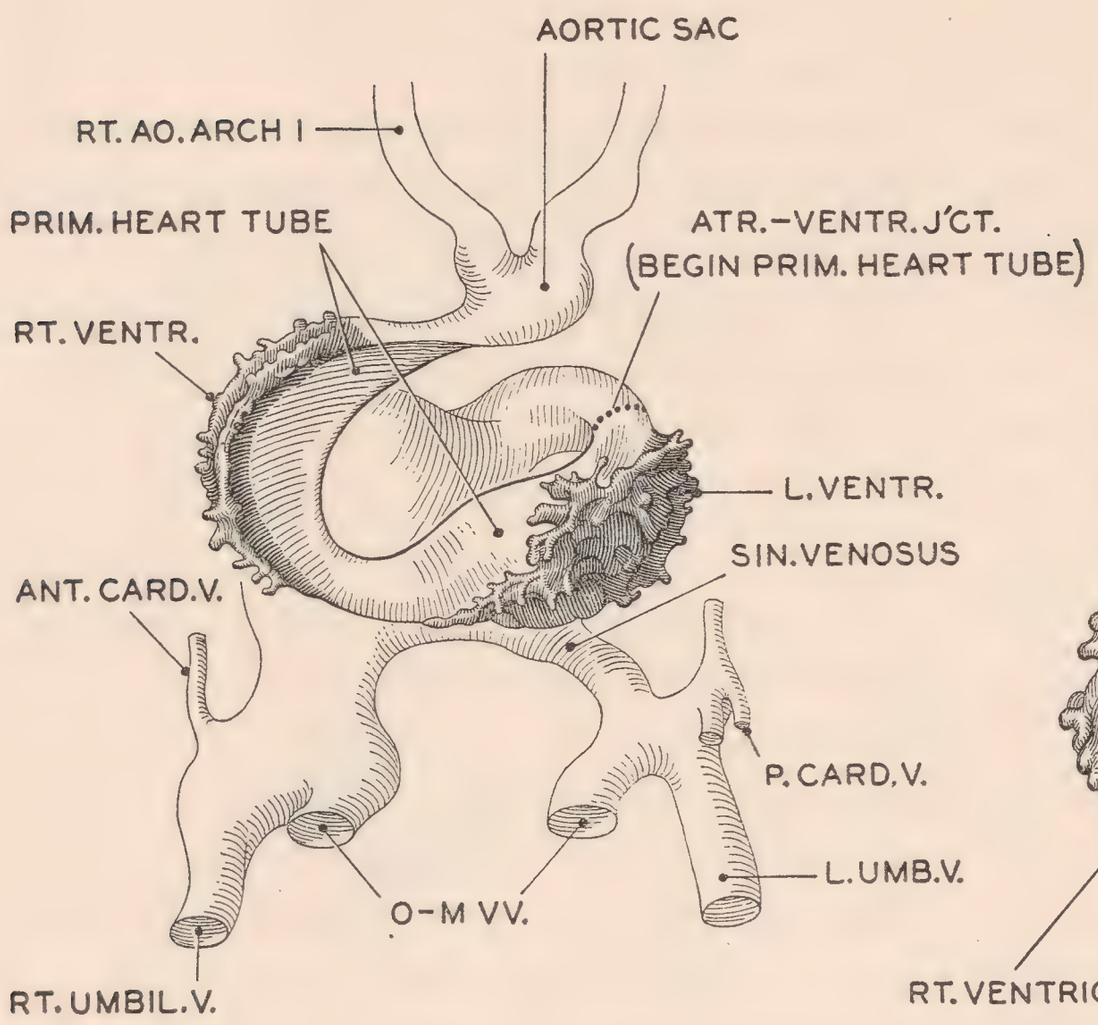
The distribution of the thick layer of gelatinous reticulum, in the age group now under consideration (horizon xv), can be seen in typical sections selected from a sagittal series shown in figure 2, xv. It still exists as a continuous sleeve around the primary heart tube, beginning at the atrioventricular canal and stopping abruptly before reaching the aortic sac. In figure 3, xv, the primary heart tube is shown at four levels of development. These drawings were made from three-dimensional reconstructions of the endothelial surfaces and are therefore the same as casts of the cavities of the heart. The form of the atria is largely influenced by the state of their contraction and consequent distention by the contained blood. The variation in the primary heart tube is less marked, and, with the gelatinous reticulum removed, its form is fairly constant for each developmental level. In passing from horizon xiii to horizon xv it becomes relatively more voluminous, and its distended parts mark what are to be the aortic and pulmonary channels.

A more striking specialization of the primary heart tube, however, one associated with the separation of the channels mentioned in the preceding paragraph, is the outgrowth of endothelial diverticulae, which occur at two sharply defined areas peripherally on the edge of the tube. These diverticulae interlock with corresponding slender trabeculae from the overlying myocardium, as was illustrated in figure 4, xiii, and figures 24 and 25, plate 3, xiii.[8] Thus in these two areas, instead of having a thick covering of gelatinous reticulum, the primary heart tube opens outward in the form of two trabeculated pouches which are the primordia of the right and left ventricles. These side pouches can be recognized as early as age group xi while the primary heart tube is a simple channel. Whether this localized growth is inherent in the endothelium itself or is induced by some activity of the embryonic myocardium can be determined only by future observations. We do know, however, that rhythmic contractions are in operation. The primitive heart tube in the embryo of age group xiii is longer, though it still shows little or no evidence of separation into two channels. By the time we reach age group xv, however, it can be seen that the bulging heart tube is definitely separating into two principal channels. And by this time, the two ventricular sacs have acquired an increased capacity. It is evident that contractions of the right ventricular sac would result in a current directed along the pulmonary border of the heart tube, whereas the left ventricular sac would favor a cross current toward the aortic border of the heart tube. It therefore seems possible that these two ventricular sacs may be the responsible factors in producing respectively the pulmonary and aortic currents. At least, the ventricular sacs are there and are functioning before the separation of the two channels takes place. We shall return to this subject in describing age group xvi.

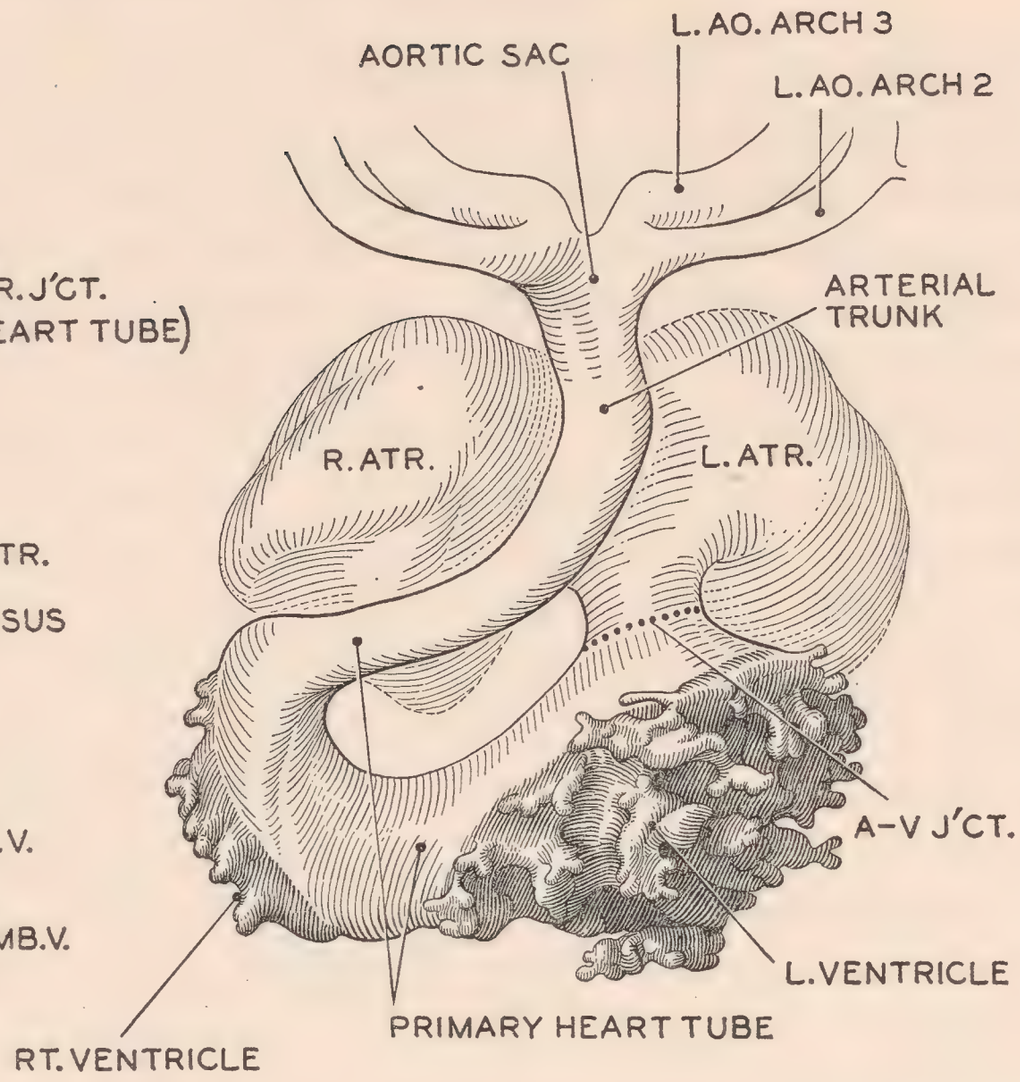
[7] C. L. Davis, The cardiac jelly of the chick embryo, Anat. Rec., vol. 27, pp. 201–202, 1924. This brief report of his observations is outstanding both in clarity and in significance.

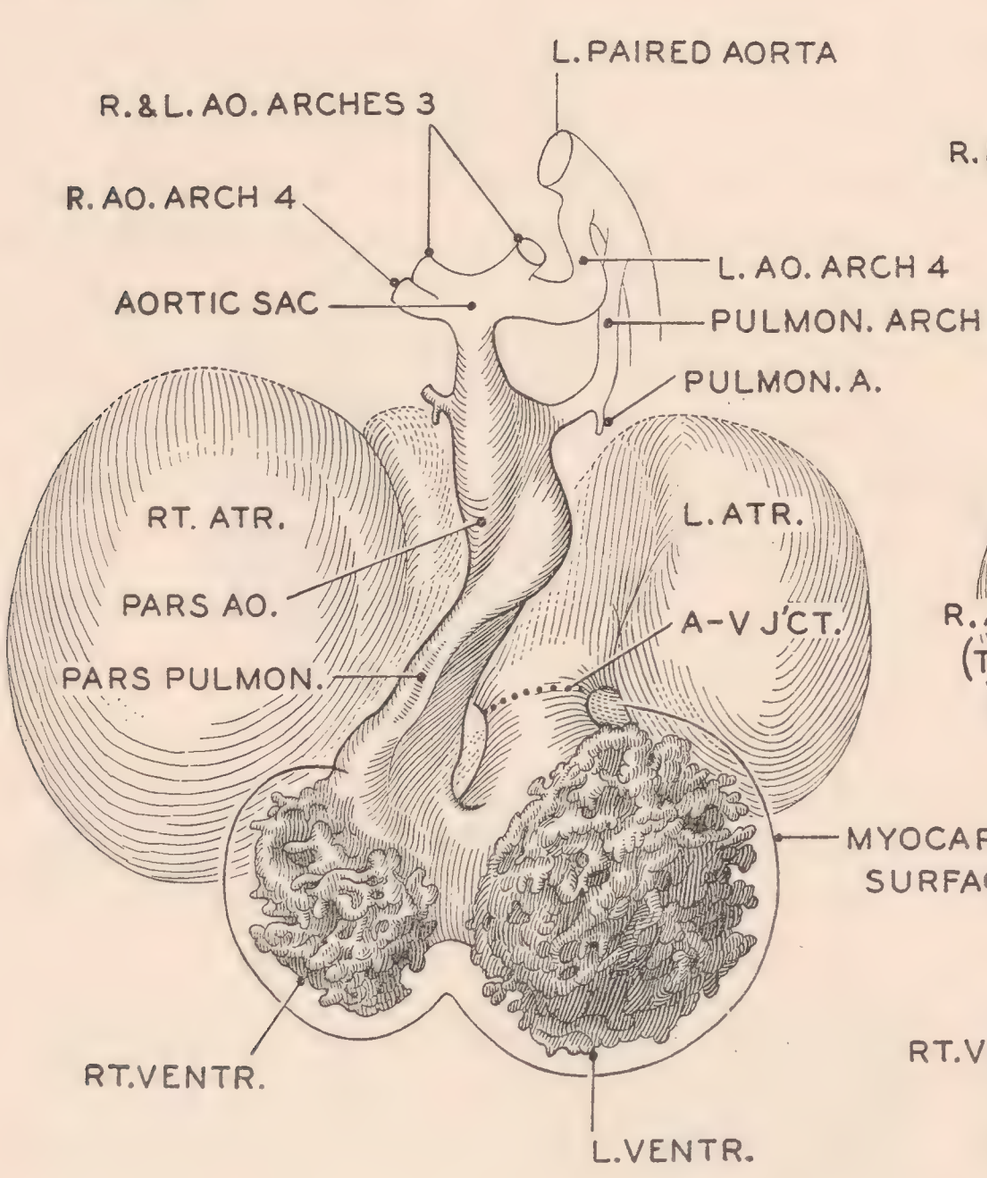
[8] G. L. Streeter, Developmental horizons in human embryos. Description of age group xiii, embryos about 4 or 5 millimeters long, and age group xiv, period of indentation of the lens vesicle, Carnegie Inst. Wash. Pub. 557, Contrib. to Embryol., vol. 31, pp. 27–63, 1945.

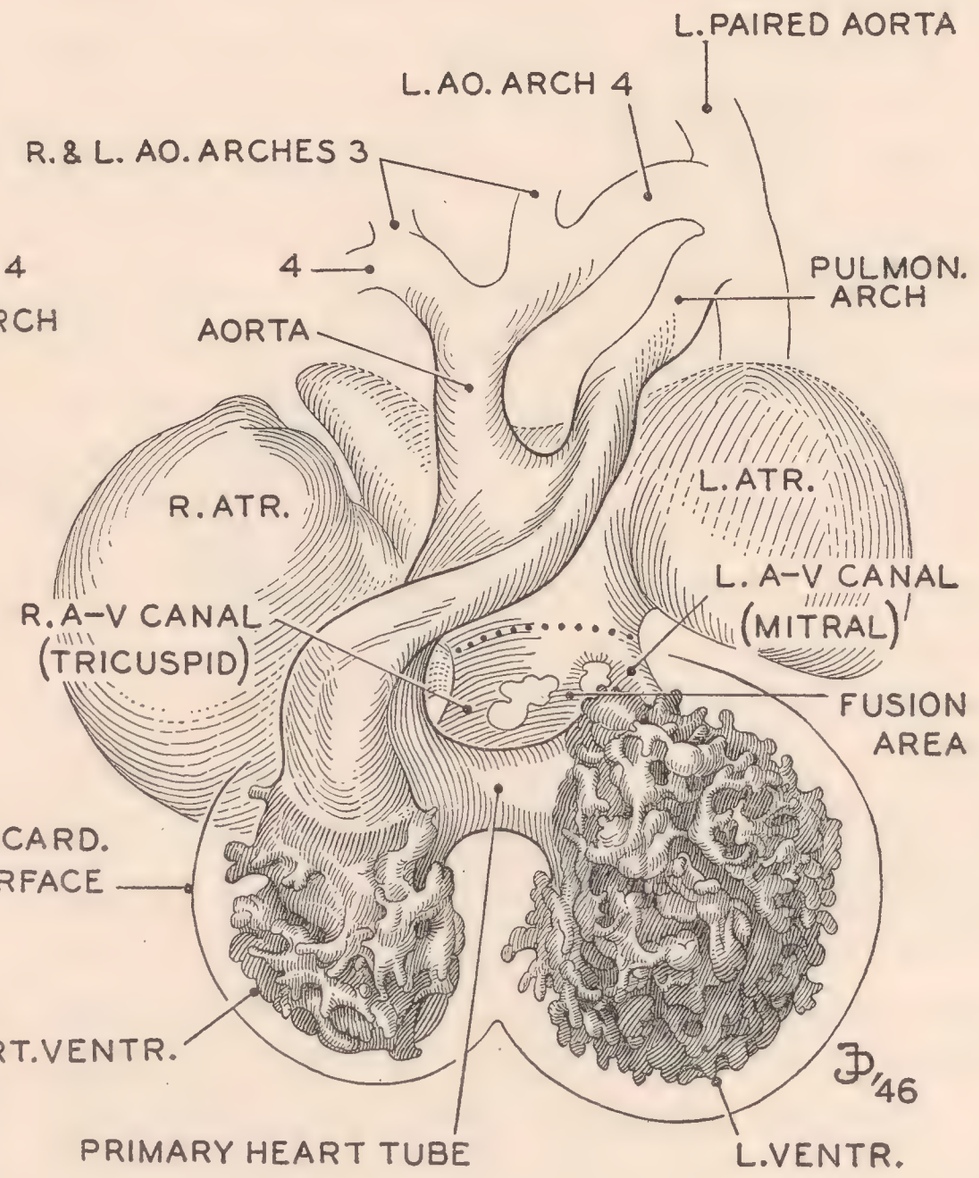
XI NO. 2053 X 60




XIII NO. 836 X 60




XV NO. 3385 X 30

XVI NO. 6510 X 30

FIG. 3, XV. Reconstructions illustrating the primary heart tube, as a part of the heart that is developmentally and histologically distinct from the atrial parts. The origin of the venous or atrial part of the heart was shown in an earlier horizon, together with the embryological relation of the atria to the primary heart tube (fig. 5, xii). The four reconstructions now before us represent only the endothelium. The cardiac jelly or gelatinous reticulum is not shown. The latter surrounds the endothelium as a supporting tissue. It is the earmark of the primary heart tube and is present throughout its length. The atria are devoid of gelatinous reticulum except at the atrioventricular junction. In two areas on the border of the heart tube the endothelium opens out as two trabeculated sacs, the right and left ventricles. Here the gelatinous reticulum is replaced by processes from the myocardium which interdigitate with the reticulated endothelium. As the ventricular sacs form, the heart tube becomes distended and gradually divided into two channels in a manner indicating separate blood currents, in accommodation to the functioning of the right and left ventricles. Drawings made by J. F. Didusch and reconstructions made by O. O. Heard. Models of age groups xi and xiii ×60; models of age groups xv and xvi ×30.

## Lung

Differing from the heart, which at this time is a functioning organ and playing a definite part in the maintenance of the life of the whole embryo, the lung as yet shows little evidence of extending its services beyond its own requirements. It is only increasing in size and becoming more specialized in form. The branching epithelial tube that constitutes its primal tissue exhibits a regulated growth, and its form, from one developmental level to the next, is determined by the rate and manner of the proliferation of its component cells. From the outset one recognizes foci of greater activity of cell proliferation. This is expressed in the bilateral symmetry of the original lung primordium. No evidence has thus far been discovered that this localized proliferation is due to special environmental influences, and until more is learned about it one must assume that it comes about through causes similar to those that initially mark off the lung area from the esophageal area, the occurrence of which, as of other marked-off areas of the gut epithelium, has already been pointed out as early as horizons xi and xii. The bilateral lung primordium is followed by a trachea with two primary bronchi, the growth foci being at their bulging tips. Then additional foci appear and produce secondary bronchi, then tertiary and so on, all in due course. The form of the trachea and primary bronchi that characterizes horizon xv is illustrated in figure 7, xv. It will be seen in that figure that, although one cannot yet speak of secondary bronchi, distinct focal swellings mark the sites where they will form.

To determine the developmental status of the lung where no reconstructions have been made, one resorts to the microscopic appearance of the sections, and important advances can be recognized in that way. The progress in histogenesis is sufficiently rapid to enable one to distinguish between horizons xiii, xiv, and xv, for instance, as is illustrated in figure 4, xv. On comparison of these three age groups it is found that in the youngest (xiii) the coelomic epithelium is still actively shedding cells which are moving in toward the gut epithelium, forming a layer that envelops the esophagus and lung bud in a common coat of visceral mesenchyme, the primordium of the vascular, muscular, and supporting tissues of these organs. In age group xiv, the shower of visceral mesenchyme has ceased and the residual coelomic epithelium is quieting down to a single-cell layer. Those cells that moved in as visceral mesenchyme begin to exhibit angiogenesis, and as this occurs the investment of the esophagus becomes marked off from the investment of the primary bronchi. The delineation of the esophageal field is aided by the circumstance that angiogenesis first occurs around its margins. It will be seen that a definite advance in differentiation of both pulmonary and esophageal epithelium is present in this age group as compared with the preceding one. Passing to age group xv, the horizon with which we are now especially concerned, both the esophageal and the bronchial capillary plexuses mark off their respective fields. Forming basket-like networks around the primary bronchi there is now a conspicuous pulmonary plexus with especially wide meshes between the bronchial tips and the atrial walls. In favorable specimens this can be seen to drain into the left atrium through a main channel, the common pulmonary vein, as shown by an arrow in specimen no. 721, figure 4, xv. What is now left of the coelomic epithelium bordering the lung surface will be the source of the visceral pleura.

## Gut Tract

As we advance from one developmental level to the next, a notable alteration in the gut tract is its continued tendency toward becoming more slender. At least, the epithelial tube comprising the primal gut becomes steadily longer, relatively to its diameter. This slenderizing process has been witnessed in the former horizons and now continues as we come to horizon xv. This is quite evident when figure 5, xv is compared with similar figures of the four preceding horizons. Among other distinctions to be mentioned is an increased definition of the several gut regions. The esophagus is relatively elongated and is now straddled from in front by the elongated primary bronchi. A ventral pancreas has made its appearance in the form of a small evagination from the under surface of the common bile duct, and at the

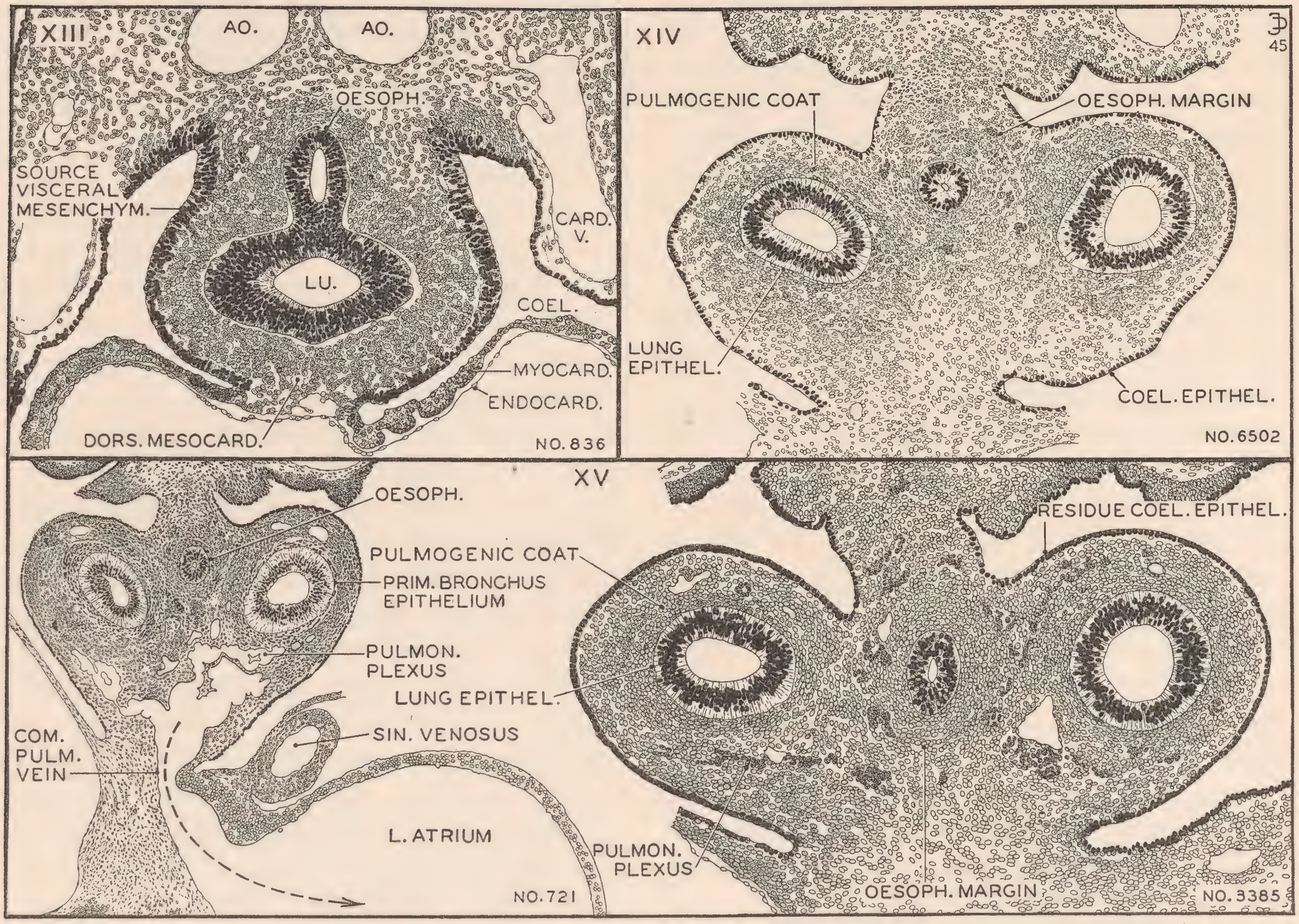



FIG. 4, XV. Degree of differentiation of the mesenchymal tissues of the lung and esophageal regions characteristic of age groups xiii, xiv, and xv. In the youngest of these (xiii) one can still see migrant cells from the proliferating coelomic epithelium. In age group xiv the coelomic epithelium has about ceased its proliferative activity and the mesenchyme is arranging itself in zones. Angioblasts are forming a net which outlines the esophagus; a photograph of this same section was shown on plate 4, xiv. In age group xv angiogenesis is taking place around the primary bronchi. Large meshes of the pulmonary plexus communicate with the common pulmonary vein, and through it the blood reaches the floor of the left atrium. This is shown in embryo no. 721, ×50. Other three sections ×100. Drawings made by J. F. Didusch.

same time the dorsal pancreas is becoming an elongated sac whose communication with the gut is reduced to a narrowing canal.

Coming to the intestinal canal, one finds a definite caecum sharply marking the junction of ileum and colon, which in the previous age group could only be approximated. The separation of bladder from rectum has passed caudal to the ureters. The elongation of the ileum has produced a definite primary intestinal loop which in horizon xiv could be spoken of only as a slight ventral deflection. It is to be remembered that figure 5, xv represents an outline of the epithelial tube only, and that this tube is surrounded throughout its length by a zone of condensed visceral mesenchyme, derived from the overlying coelomic epithelium, and with advanced angiogenesis approximately like the condition shown in figure 4, xv, embryo no. 3385.

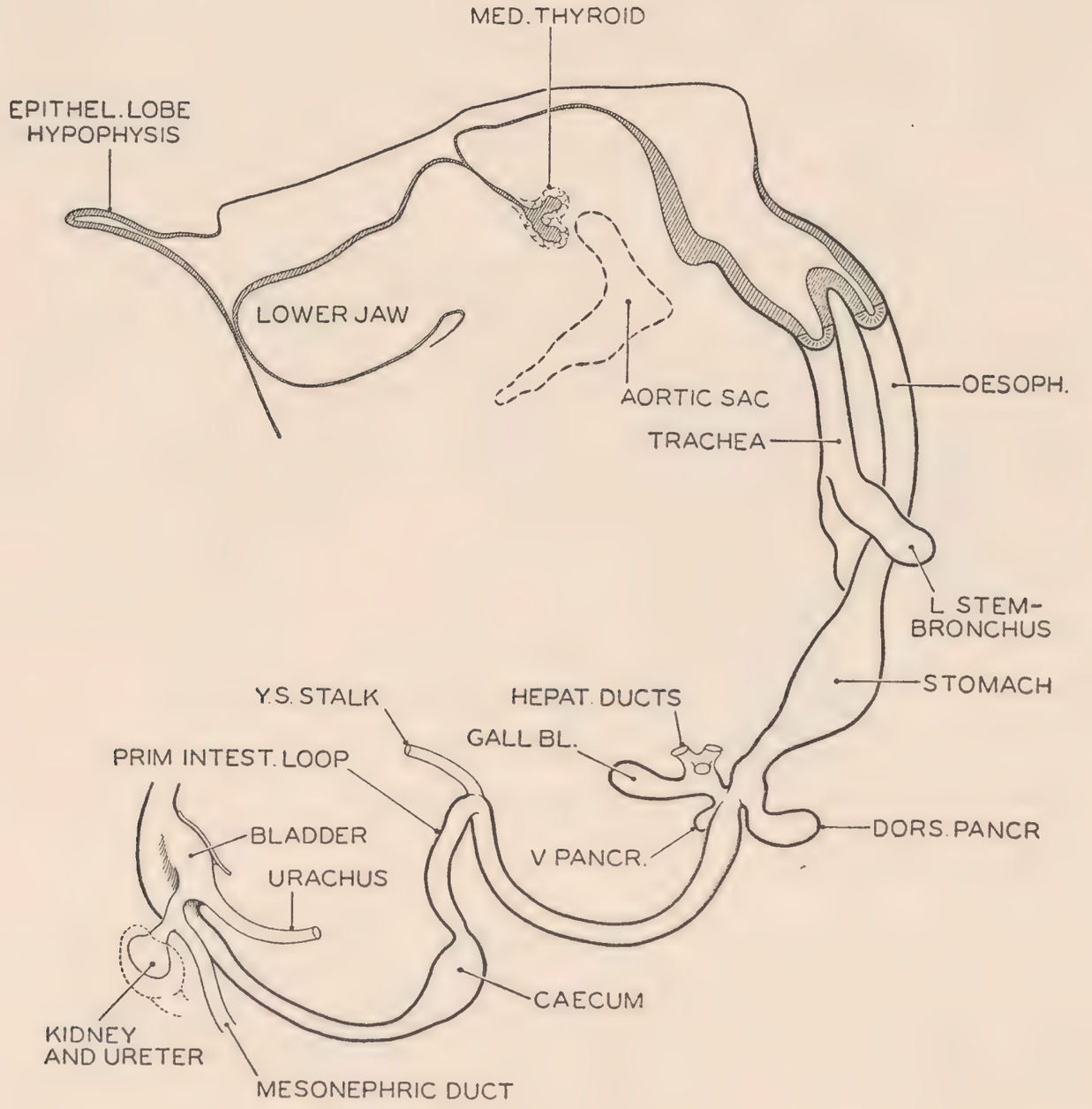



FIG. 5, XV. Drawing of a three-dimensional reconstruction of the epithelial tract of the gut system and associated structures, typical of age group xv, embryo no. 6504. The pharynx is shown in median sagittal section. Drawing made by J. F. Didusch. Reconstruction made by O. O. Heard. ×33.4.

Finally, it is to be noted that the median thyroid is still connected to the epithelium of the floor of the mouth by an elongated slender strand, whereas the gland with its surrounding reticulum rests near the oral border of the aortic sac. The hypophysis lies rostral to the ridge of the oral membrane.

## CENTRAL NERVOUS SYSTEM

In the four horizons comprising the embryos surveyed in this issue, the sketches of the brain are shown at a uniform magnification of 12.5 diameters, and thus they can be directly compared with one another. It was necessary to step down the enlargement from that used in earlier horizons, but the reader can compensate for this by remembering that the brain shown in figure 6, xv is actually about one-third larger in its over-all dimensions than the brain of the preceding horizon shown in figure 4, xiv, in the last issue. It is the difference in size that at once distinguishes the brains of the xvth from those of the xivth develop-

mental level. There are in addition, however, other less obvious characters which mark advances in the differentiation of the brain wall of the age group we are now considering.

The rhombic grooves, associated with the origin of the nerves to the pharyngeal bars and to the mandibular and maxillary masses, retain their primal characteristics. As the basal plate grows relatively larger, we shall see it, in the following age groups, spread forward and become continuous with the tegmentum. In this process the rhombic grooves are crowded

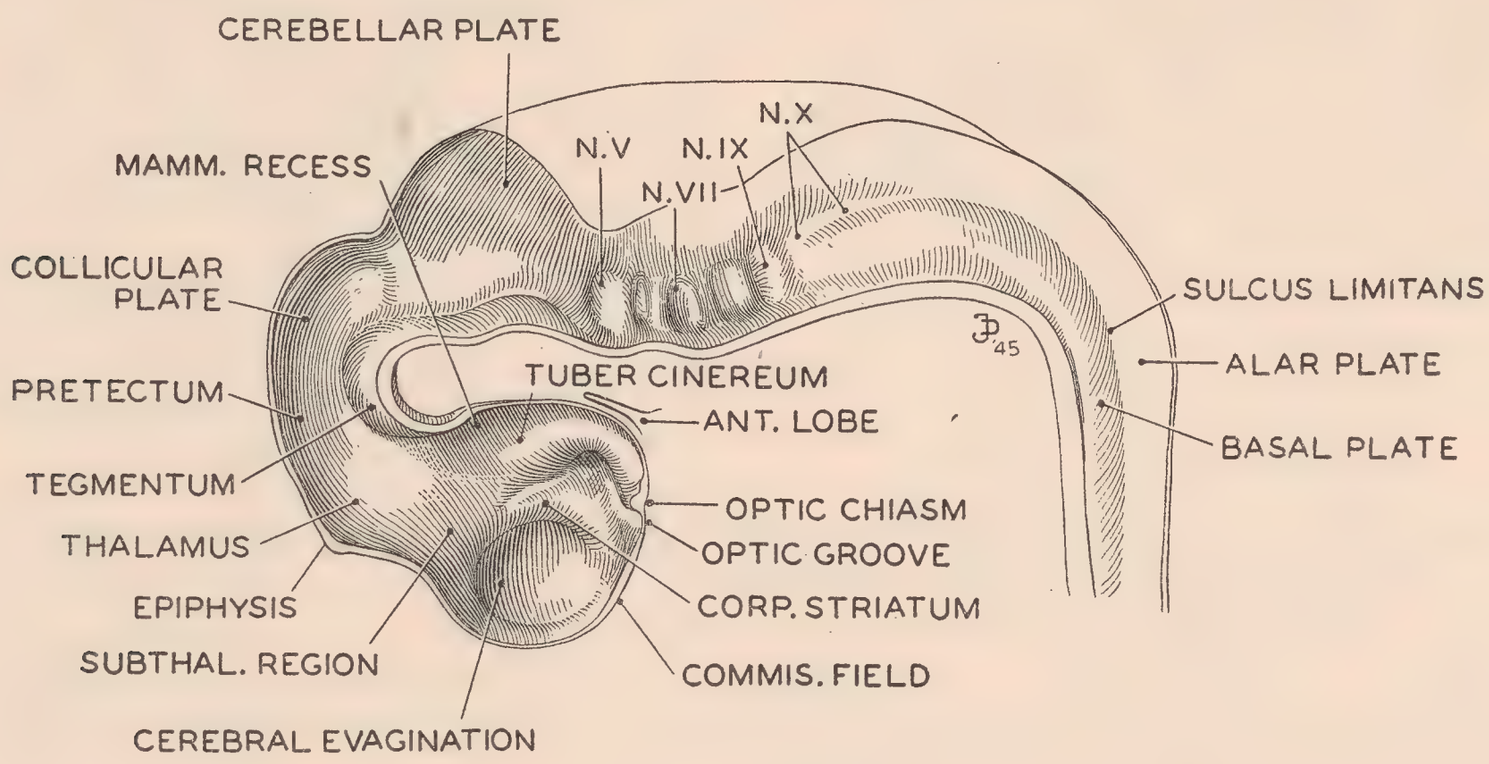



FIG. 6, XV. Three-dimensional reconstruction of the brain of embryo no. 3385, typical of age group xv. The model is sectioned in the median sagittal plane and thus displays the inner markings of the neural tube. Comparison of this sketch with those of the preceding age group (shown in fig. 4, xiv) shows some advance in definition of neural parts. The two mid-line dips in the floor opposite the trigeminal and facial grooves are still present. Drawing made by J. F. Didusch. Reconstruction made by O. O. Heard. ×12.5.

lateralward and disappear in the sulcus limitans. It can be seen that this actively growing basal plate is a cranial continuation of the basal plate of the spinal cord and, like it, is giving origin to a mantle zone and the beginning of the reticular formation. The nuclei of the hypoglossal nerve, which are distinct at this time, are the counterparts of the ventral horns and spinal ventral nerve roots.

In the rostral part of the neural tube the primordium of the epiphysis can be distinguished as a slight mid-line thickening of the roof in the thalamic level. The cerebral evagination is now becoming definite, whereas before one could only identify the region where it was to occur. At the same time the corpus striatum becomes a prominent fold. The site of the optic evagination is becoming compressed as a slit between the corpus striatum and the optic chiasm mass. From this slit a thin strip of wall extends to the mid-line, forming the optic groove (or preoptic recess), which constitutes a landmark between the striated commissural thickening and the thickened ridge through which the optic chiasmal fibers will pass when they arrive at the mid-line. It is to be noted that in the design of the brain wall the path for cross-ing is laid down both for the optic decussation and for the anterior commissure before the nerve fibers themselves are present. One cannot speak of a hypophysis yet, beyond the presence of the pocket of pharyngeal epithelium that will form its anterior lobe. The origin of this pocket in front of the oral membrane is shown in figure 5, xv. The neural lobe makes its appearance in the next age group.

The special sense organs (eye and ear) will be taken up in more detail in subsequent age groups. Consideration of them at this time will be limited to the brief references made in the following summary of this horizon.

## SUMMARY OF HORIZON XV

As a summary of their inner structure, outline drawings of selected organs of embryos found in this age group are shown in the accompanying figure 7, xv. Organs were selected which could be easily recorded in this manner from serial sections. Where active changes are taking place, both younger and

older representatives are illustrated. Thus the figure presents a syndrome of characters which an embryo must possess to qualify as a member of this horizon, and at the same time it records the amount of variation in development found within the group. Similar syndromes are illustrated in the succeeding three horizons, and should be compared with one another. In the case of the eye, an arbitrary border line for the beginning of this age group was set at the time of closure of the lens vesicle, and the beginning of the next group was set at the appearance of pigment in the outer layer of the retina, sufficiently distinct to be recognized with an 8-mm. objective. The eyes of lower and upper borderline specimens are shown in the figure under discussion. Every specimen listed in our table of embryos as belonging in horizon xv meets these requirements. One representative of the ear vesicle serves as typical for the group. It has a distinct endolymphatic appendage, differentiated and set off from the remainder of the auditory sac. This appendage, however, remains relatively short. The olfactory placode can likewise be represented by one example. It shows little evidence as yet of the part it is to play in the establishment of a respiratory by-pass. Three representatives illustrate the form of the epithelial lung: one on the younger border line, one on the older border line, and one intermediate. The outlines of the trachea and primary, or stem, bronchi were obtained by geometric projections. It will be noted that the sites of the secondary bronchi vary

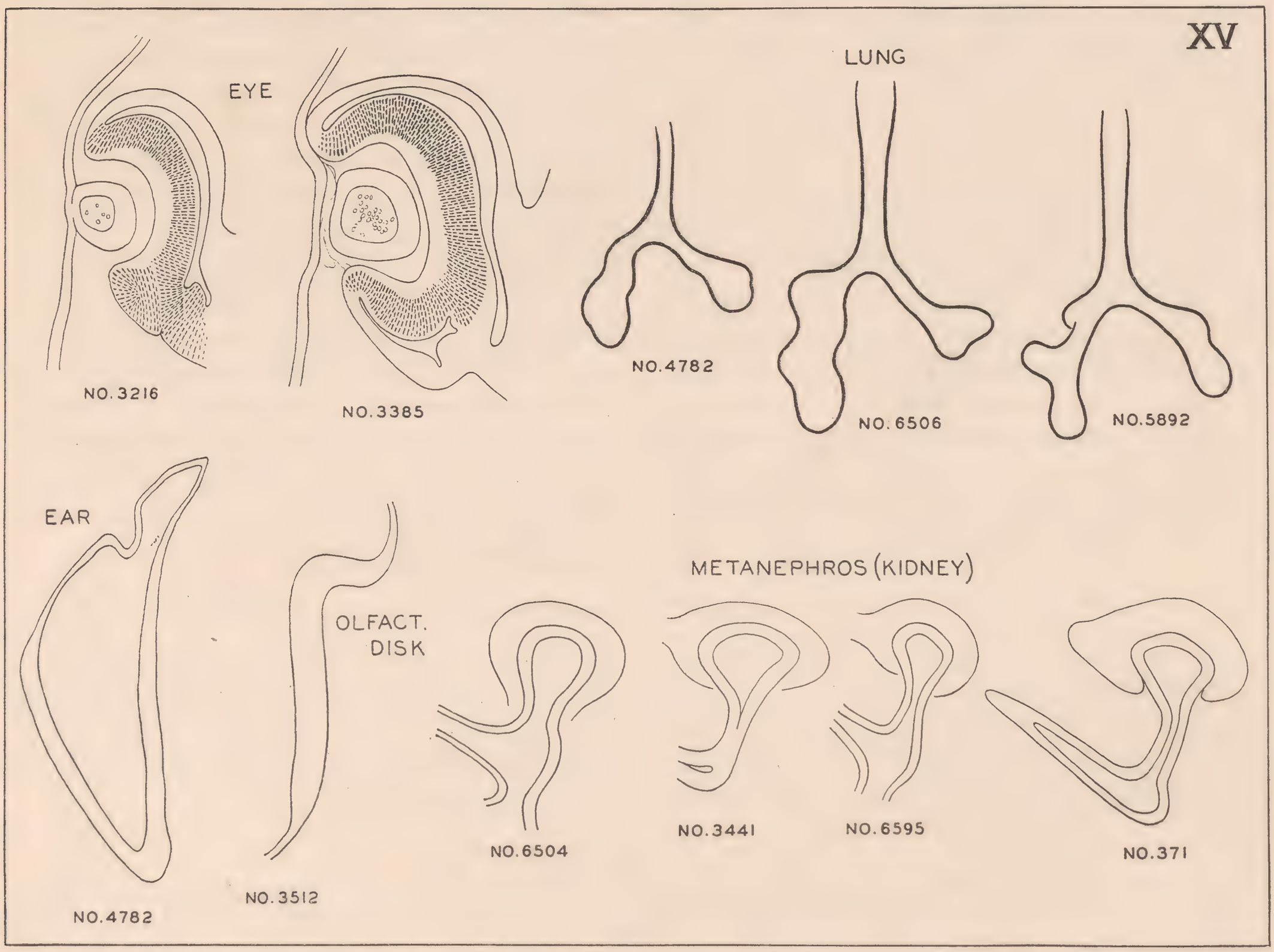



FIG. 7, xv. Syndrome of structural characters typical of age group xv. In case of the eye, lung, and kidney, examples are shown of younger and older members of the group. If an embryo is to be classed in this horizon, it must fit within such ranges. In any event the eye lens must be closed, though in younger specimens it may still be attached to the surface epithelium, or in older ones it may be completely detached. The olfactory placode is quite variable in form, depending on the plane of section. This reduces its value as an index of development. Bronchial trees ×40; all other organs ×about 80.

from slight swellings in the younger members to short, stubby evaginations in the older ones. By this time the primary bronchi extend dorsally astride the esophagus. The kidney and ureter are shown in four degrees of development as found from younger to older members. The length of the ureter appears to be the best criterion of advancement. But evidence of this, as of the width of its lumen and its origin from the Wolffian duct, is largely influenced by the chance plane of section. The same factor is to be taken into account in studying the form of the nephrogenic capsule.

In addition to the structural features illustrated in figure 7, xv, one must also keep in mind some of the definitive characters met with in the heart, gut tract, and central nervous system, which have been surveyed in the preceding pages in greater detail. The heart at this time produces a satisfactory circulation by means of the primary heart tube, encased in a covering of gelatinous reticulum that, in the absence of valves, serves as a compression cushion, valvelike in its operation. From the ventral border of the primary heart tube, two trabeculated pockets of endocardium spread outward to interdigitate with the overlying myocardium. These are to be the right and left ventricles. Coincident with these, one begins to have evidence in the form of the heart tube of two different currents in the contained blood stream, pulmonary and aortic currents.

In the gut tract one finds the gut epithelium still marking off its main subdivisions, and there is now a definite ileocaecal junction, a primary intestinal loop, and a ventral pancreas. In the region of the esophagus and trachea, angiogenesis has advanced in such a way as to mark out definitely the fields of visceral mesenchyme that belong to these respective structures. Finally, in the central nervous system one can now definitely locate the epiphysis, although there is still lacking a neural lobe for the hypophysis. Also the lateral evaginations of the two hemispheres have acquired definite contours with associated accentuation of the corpora striata. Corresponding to the prominence of the pharyngeal bars that still characterizes these embryos, the rhombic grooves, associated with their nerve supply, remain as a conspicuous feature of the floor of the hindbrain.

## Normal Embryos in Carnegie Collection Belonging in Horizon XV

As in previous horizons, the embryos qualifying as members of this age group are listed in the following table, and the same explanatory notes, as especially noted in the table for horizon xiii, are to be applied here. In some cases there is reason to think that the recorded size is due to abnormal circumstances and that in this respect the specimens are not comparable to the others of the list. Therefore, where possible, a corrected, or estimated, length is substituted and is indicated by an asterisk (*).

### List of Embryos

| Serial no. | Size (mm.) | Grade | Fixation | Cutting medium | Plane | Thinness (microns) | Stain | Date, donor, and remarks |
|---|---|---|---|---|---|---|---|---|
| 2.. | E., 7.0<br>Ov., 25 × 25 × 25 | Good | Alc. | P | Trans. | 15 | Al. carm. | 1888, C. O. Miller, Newmarket, Va.<br>Among youngest in group |
| 241.. | E., 6.0<br>Ov., ..... | Good | Formol | P | Trans. | 10 | H. Congo red | 1904, W. C. Sandrock, Baltimore |
| 371.. | E., 6.6<br>Ov., ..... | Good | Formol | P | Sag. | 10 | Al. coch. | 1913, M. Brödel, Baltimore<br>Shrunken and cracked in embedding |
| 721.. | E., 9.0<br>Ov., 30 × 20 × 10 | Exc. | Zenker formol | P | Trans. | 15 | H.-E. | 1913, unknown physician, Chicago<br>Median place in group |
| 810.. | E., 7.0<br>Ov., 30 × 25 × 15 | Good | Alc. | P | Sag. | 20 | Al. coch. | 1913, H. P. Cole, Mobile, Ala. |
| 1006.. | E., 9.0<br>Ov., 37 × 26 × 22 | Poor | Formol | P | Frontal | 20 | H.-E. or. G. | 1914, J. Morris Slemons, Berkeley, Calif.<br>Among older members of group; operative |

(*Continued on following page*)

LIST OF EMBRYOS—*Continued*

| Serial no. | Size (mm.) | Grade | Fixation | Cutting medium | Plane | Thinness (microns) | Stain | Date, donor, and remarks |
|---|---|---|---|---|---|---|---|---|
| 1091.. | E., 7.2<br>Ov., 28 × 26 × 20 | Poor | Formol | P | Frontal | 20 | Al. coch. | 1915, W. H. Houston, Fishing Creek, Md.<br>Tissues macerated |
| 1354.. | E., 7.8<br>Ov., 35 × 30 × 25 | Good | Formol | P | Sag. | 20 | Al. coch. | 1916, W. F. Twigg, Cumberland, Md.<br>Tissues excellent; in youngest third of group |
| 1767.. | E., 11.0<br>Ov., 41 × 23 × 5 | Good | Formol | P | Sag. | 40 | H.-E. or. G. | 1917, H. J. Boldt, New York<br>In oldest third of group |
| 2743.. | E., 7.2<br>Ov., 19 × 18 × 14 | Poor | Formol | P | Trans. | 20 | Al. coch. | 1919, B. M. Cissel, Baltimore<br>Among youngest members; macerated |
| 3216.. | E., 6.5<br>Ov., 30 × 30 × 5 | Good | Formol | P | Trans. | 20 | Al. coch. | 1920, M. W. Lyon, Jr., South Bend., Ind.<br>Hysterectomy; in youngest third of group |
| 3385.. | E., 8.3<br>Ov., 25 × 20 × 16 | Exc. | Corros. acetic | P | Trans. | 20 | H.-E. or. G. | 1921, W. L. Millea, Baltimore<br>In oldest third of group; some sections lost |
| 3441.. | E., 8.0<br>Ov., 25 × 24 × 20 | Good | Formol | P | Sag. | 10 | Al. coch. | 1921, G. C. McCormick, Sparrows Pt., Md.<br>Plate 1, xv |
| 3512.. | E., 8.5<br>Ov., 33 × 28 × 25 | Good | Formol | P | Trans. | 10 | Al. coch. | 1921, A. L. Peck, West Palm Beach, Fla.<br>Special photographs, plate 1, xv |
| 3952.. | E., 6.7<br>Ov., 30 × 25 × 15 | Good | Formol | P | Frontal | 15 | Al. coch. | 1922, H. D. Senior, New York<br>Median place in group |
| 4602.. | E., 9.3<br>Ov., 33 × 30 × 26 | Good | Formol | P | Sag. | 15 | Al. coch. | 1924, Karl M. Wilson, Baltimore<br>Therapeutic abortion; tissues excellent |
| 4782.. | E., 9.0<br>Ov., 14 × 13 × 11 | Poor | Formol | P | Frontal | 20 | Al. coch. | 1924, W. L. Millea, Baltimore<br>Histology poor |
| 5892.. | E., 7.3<br>Ov., ..... | Good | Corros. acetic | C-P | Frontal | 10 | Al. coch. phloxine | 1929, V. Danchakoff, New York<br>Transitional to next older group |
| 6223.. | E., 6.7<br>Ov., ..... | Poor | Alc. | C-P | Sag. | 8 | 0 | 1930, R. Enders, Baltimore<br>Good photographs; sections fragmented, not saved |
| 6504.. | E., 7.5*<br>Ov., ..... | Exc. | ..... | C-P | Sag. | 6 | Al. coch. | Good for early ureter; one of the youngest in group |
| 6506.. | E., 7.5*<br>Ov., ..... | Exc. | ..... | C-P | Frontal | 10 | Al. coch. | Favorably cut for mesonephros, ureters, and eye; in oldest third of group |
| 6508.. | E., 7.3*<br>Ov., ..... | Good | Corros. acetic | C-P | Trans. | 6 | Al. coch. | Excellent for early gonad; older member of group; sections cracked and many lost |
| 6595.. | E., 8.0<br>Ov., ..... | Good | Formol | C-P | Sag. | 10 | H.-E. | 1932, L. Getz, Panama<br>Nervous system poor; some tissues excellent |
| 7199.. | E., 8.4<br>Ov., ..... | Good | Formol | C-P | Frontal | 10 | Al. coch. phloxine | 1935, K. H. Martzloff, Portland, Ore.<br>Operative; fixed few seconds after removal |
| 7364.. | E., 9.5<br>Ov., 35 × 30 × 25 | Poor | Formol | C-P | Frontal | 8 | H.-E. | 1936, C. W. Lawson, Glasgow, Mont.<br>Macerated; median in group |

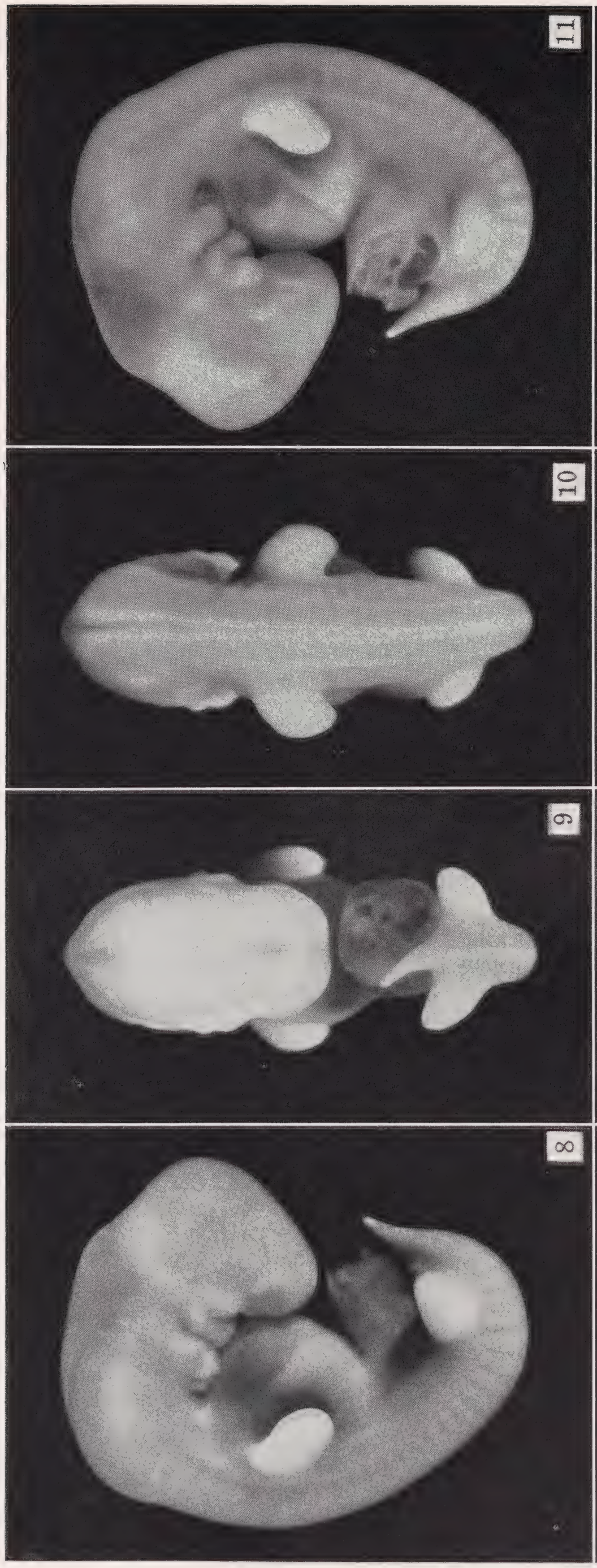
## Plate 1, xv

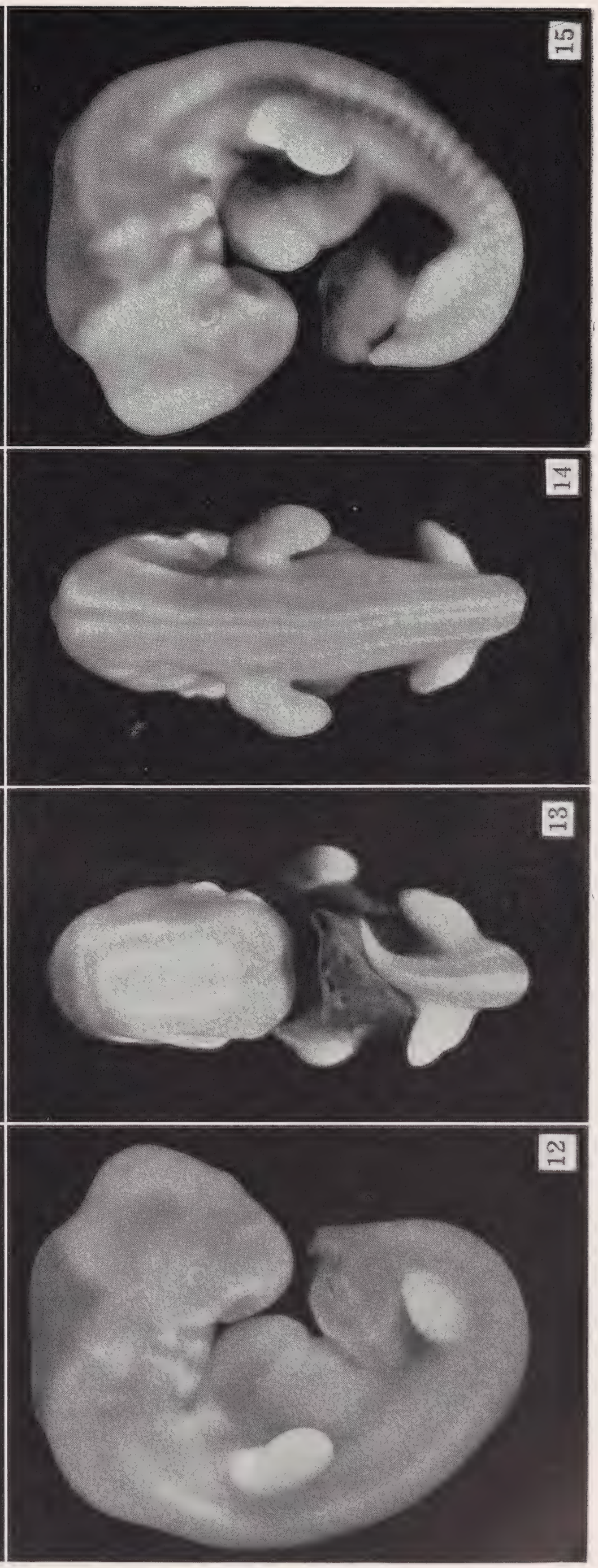
The four views of each of two embryos shown on this plate illustrate the respective characteristics of younger and older members of age group xv. In the younger specimen (no. 3441) the hand plate is marked off from the arm, and the distinction is more pronounced in the older one (no. 3512). When filled with blood the marginal vein can be seen. The leg bud is larger and rounded opposite the lumbar levels, and more tapering opposite the sacral levels. The tip of the latter will form the foot (figs. 12, 13; also see fig. 1, xvi). The somites and nerve ganglia can be seen distinctly from the occipital region to the caudal end of the spine. The head region, being the oldest part of the body, shows the most advanced characters. The olfactory placode, a shallow depression, is visible in profile view. A subdivision of the maxillary growth center forms the lateral border of the nasomaxillary groove. In all the profile views on this plate one can distinguish the trigeminal nerve and its branches. On the mandibular bar one can distinguish the auricular hillocks that are to form the crus and tragus of the external ear. The hyoid bar with its three hillocks is large and partly crowds over onto the third pharyngeal bar. The latter can be seen in figure 15 with a depression behind it, known as the cervical sinus. The swelling closing around this is the primordium of cervical muscles and associated tissues that are to spread down over the heart, making the thoracic wall. At this time most of the brain is very thinly covered. It is only around the pharyngeal bars, eye, and olfactory placode that one finds skull elements being laid down. In figures 12 and 15 one sees the form of the ear vesicle.

It should be stated here that, with but one exception, the photographs of embryos shown on the twelve plates of this issue were made by C. F. Reather, of the Carnegie technical staff. The fidelity with which they portray these difficult objects is to be attributed to his skill and painstaking efforts.

Figs. 8–11. No. 3441, ×8.

Figs. 12–15. No. 3512, ×8.

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## Plate 2, xv

All the embryos shown on this plate have the essential morphological characteristics of those that were seen on the preceding plate, and all belong in age group xv. The variations here are those due to differences in fixation, shrinkage, and original condition of the specimen. The embryos shown in the upper row (figs. 16–18) were stained in alum cochineal to portray the surface markings better. The form of the limb buds is one of the most constant characters, but it may be misleading because of illumination. All these specimens have been cut in serial sections and their inner structure verified as qualifying them as members of this age group. Where there is evident shrinkage, this has in part been compensated for by using more enlargement. The numbers and enlargement of the embryos are as follows:

Fig. 16. No. 3216, ×9.
Fig. 17. No. 3216, ×9.
Fig. 18. No. 810, ×8.
Fig. 19. No. 6595, ×8.
Fig. 20. No. 3952, ×8.
Fig. 21. No. 5892, ×8.
Fig. 22. No. 7199, ×7.
Fig. 23. No. 3385, ×8.
Fig. 24. No. 6506, ×8.

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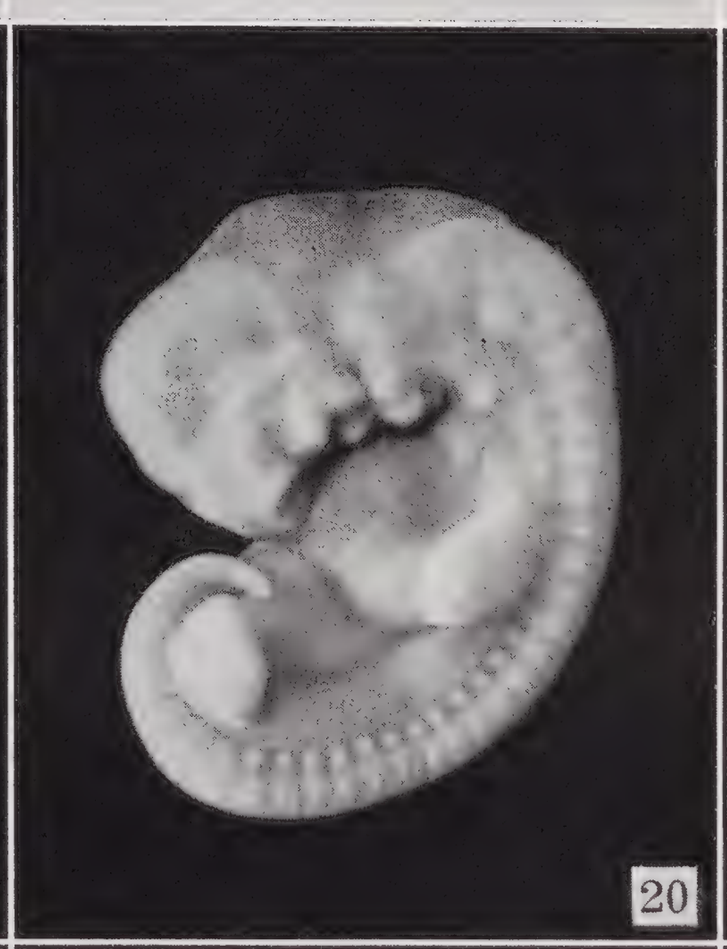
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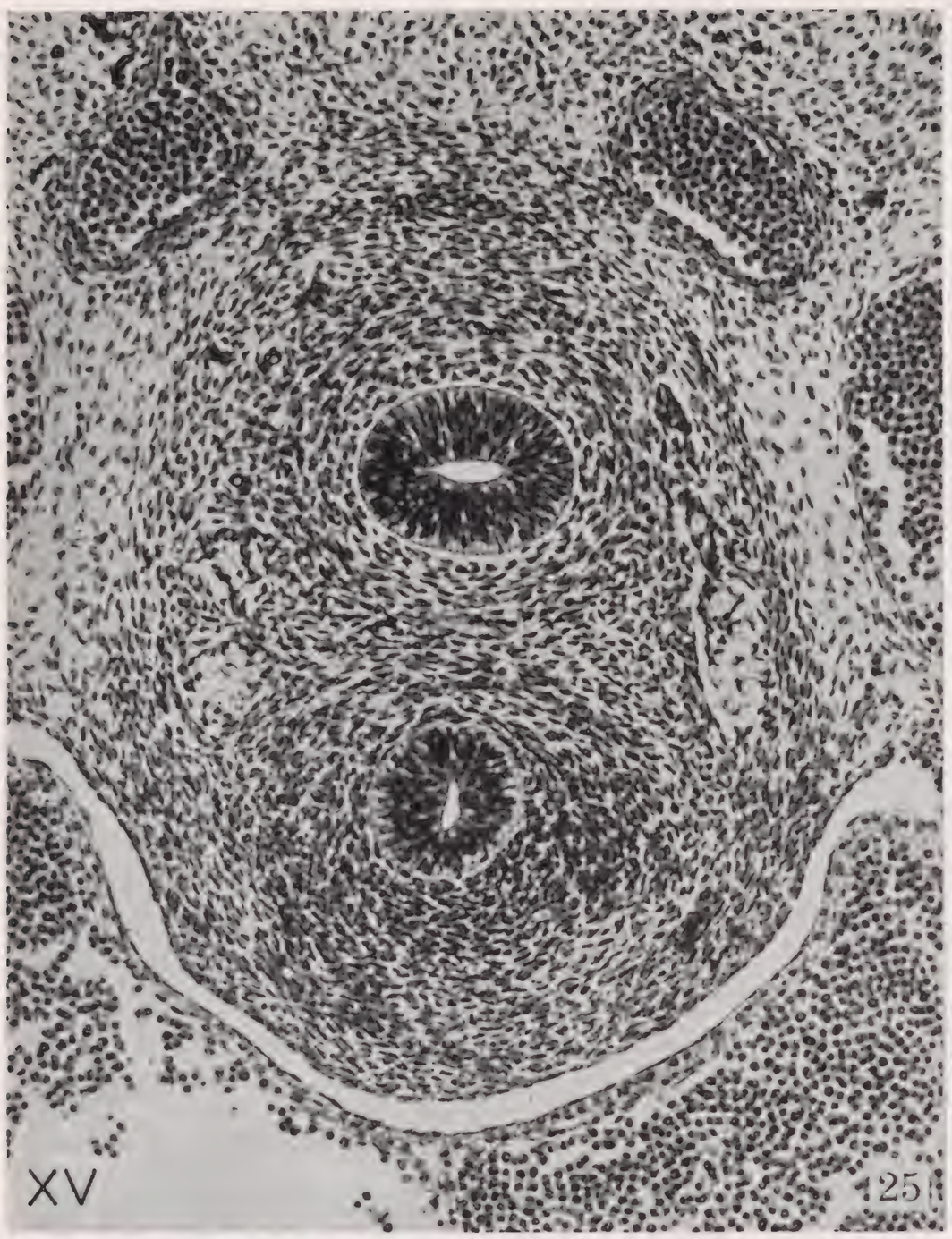
## Plate 3, xv

The four sections on this plate are taken from comparable levels through the esophagus and trachea, and were chosen as typical of the four horizons xv to xviii: fig. 25, embryo no. 3512, section 12-4-6; fig. 26, embryo no. 6517, section 21-4-3; fig. 27, embryo no. 6521, section 30-3-5; fig. 28, embryo no. 4430, section 17-4-5. All are shown at the same enlargement, ×150. In each instance there is an increase in size of the lumen, especially of the trachea. There is also notable progress in the specialization of the epithelium and in the histogenesis of the structures surrounding it. In the oldest (fig. 28) one sees, above and to the right, the thick wall of the aorta. In the top center is epithelial esophagus surrounded by a wide submucosal zone, and outside that is the primordium of the muscular coats. Between, and lateral to, the esophagus and trachea is the very large right vagus nerve. Only the edge of the left vagus can be seen. The trachea differs markedly from the esophagus, having a dense combined connective-tissue and cartilaginous primordial wall. At the bottom are the two pulmonary arteries. These various structures can be traced backward from step to step, and can be identified even in the youngest horizon (xv).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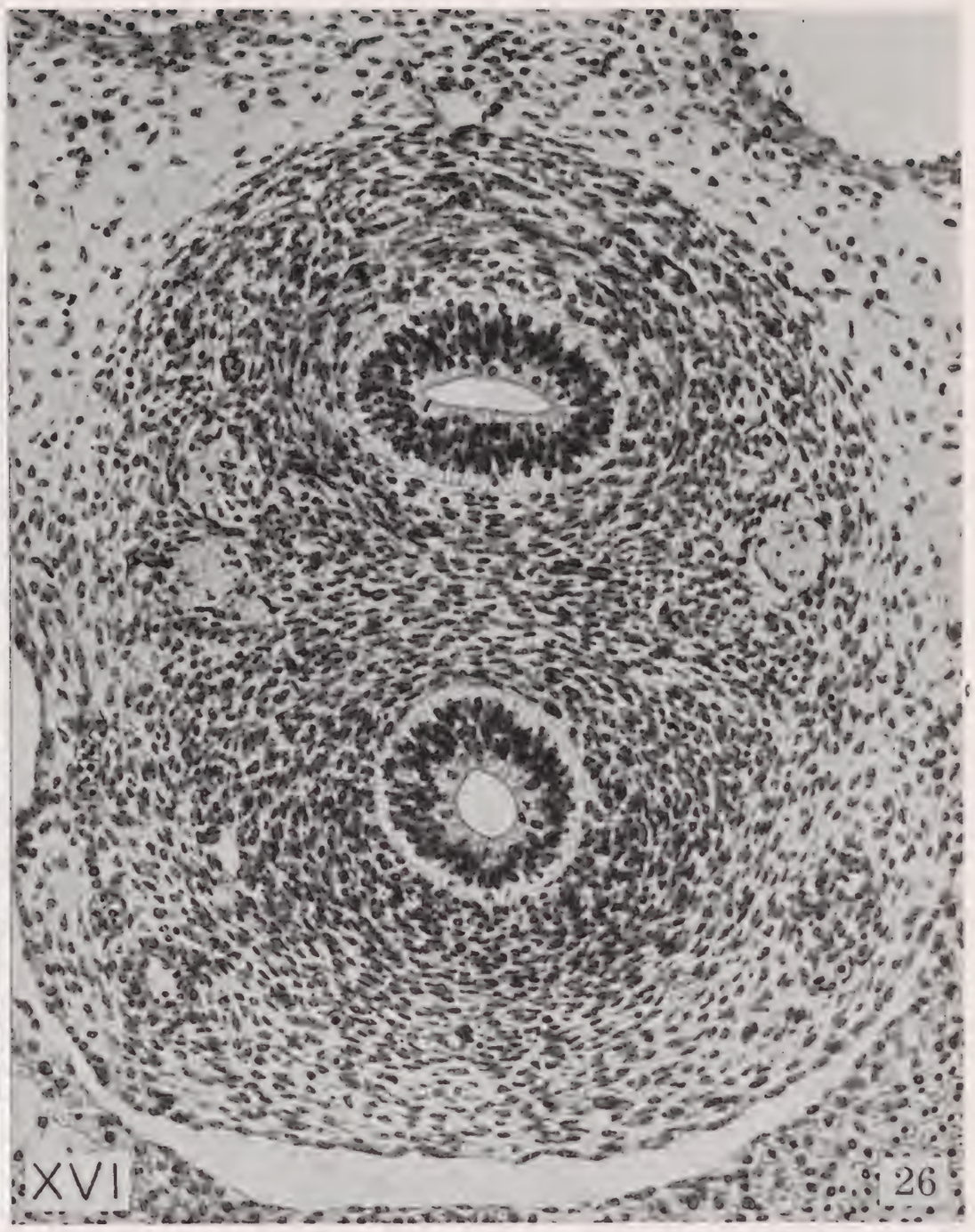
XVI
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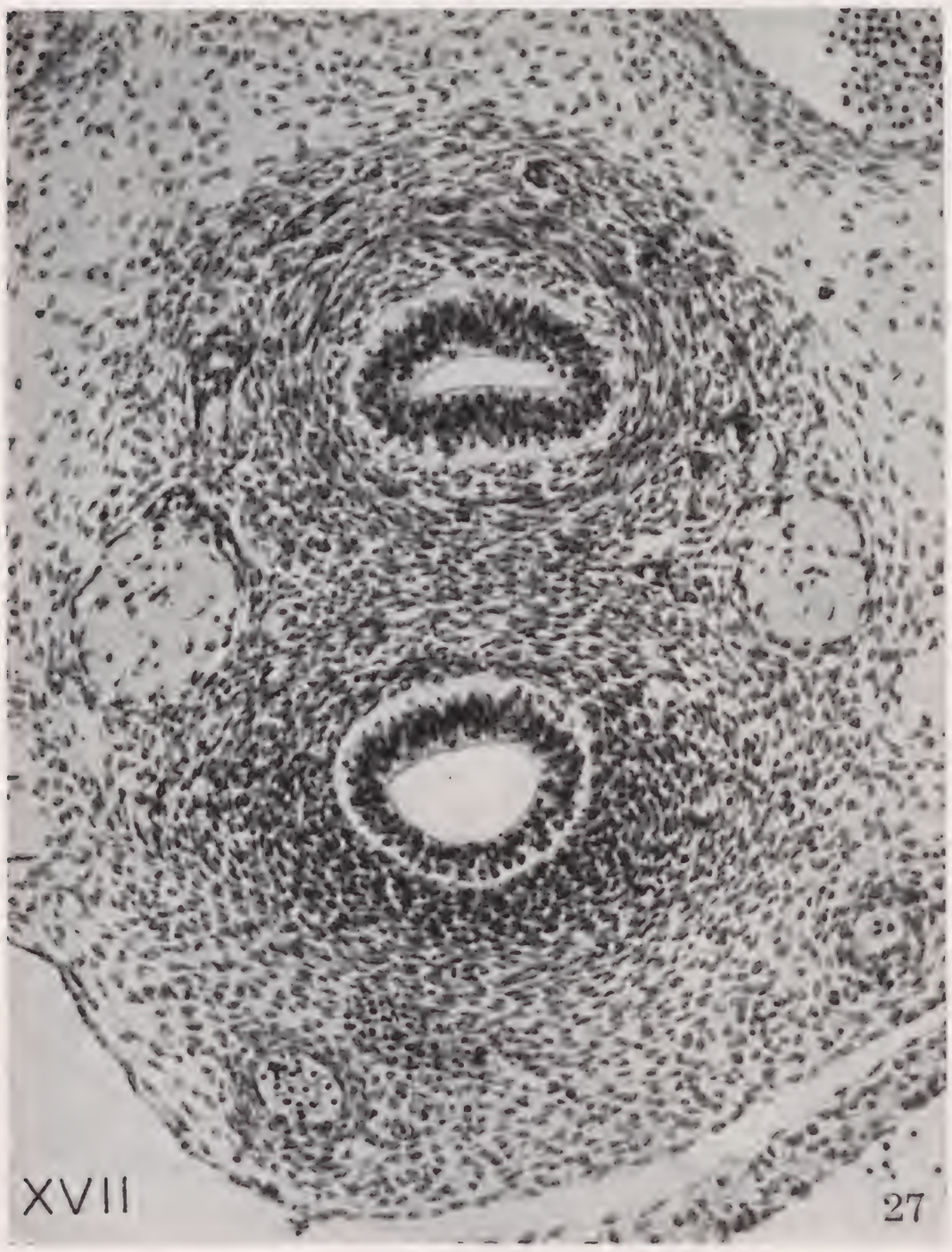
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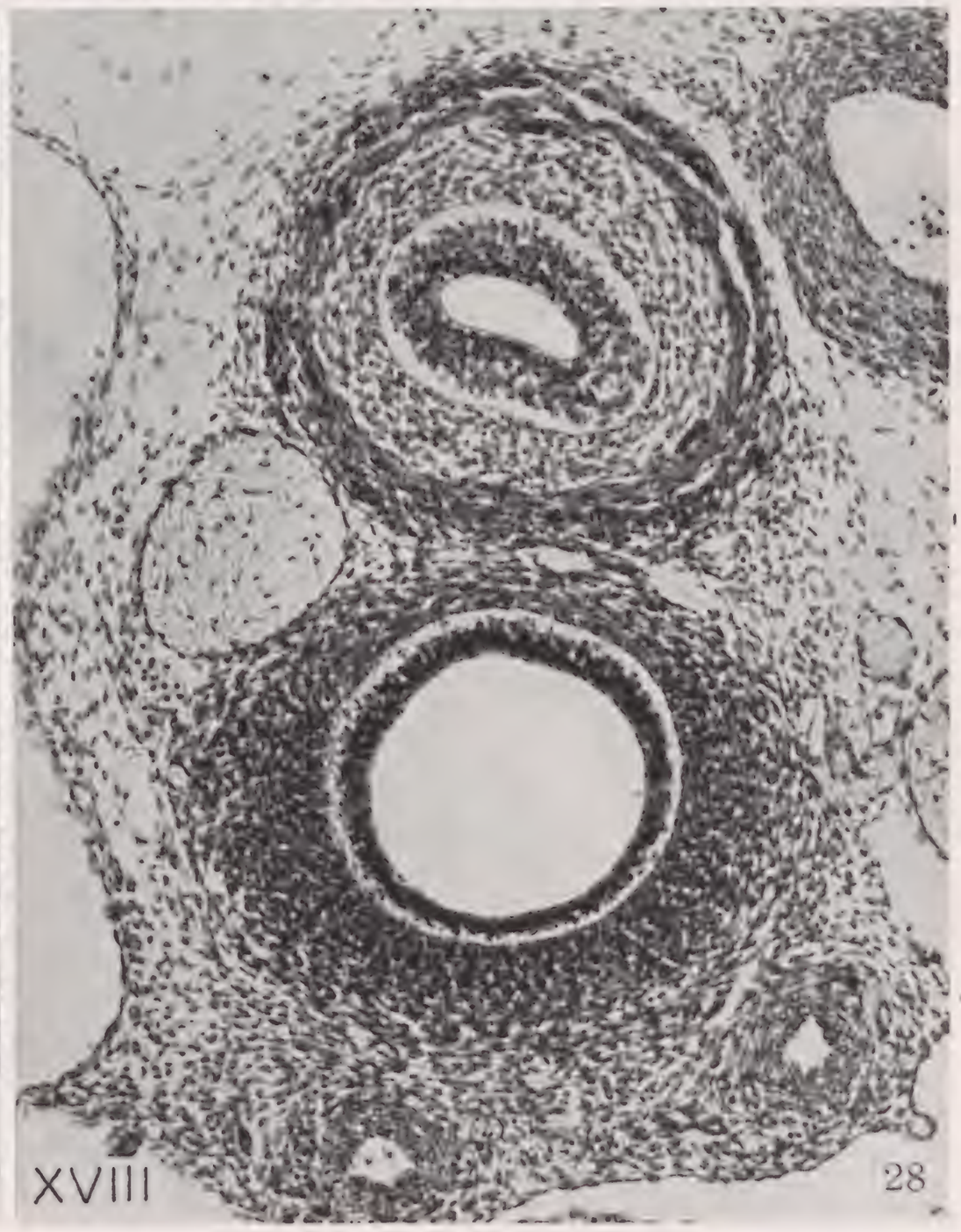
XVIII
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## HORIZON XVI

### External Form, Size, and Age

The general proportions and shape of the head of the embryos composing this age group are illustrated on plates 1–3, xvi. It will be seen that these are fairly uniform, owing in large measure to the brain, whose main parts can be recognized in the surface modeling of the head. The collicular and thalamic regions are relatively longer and more prominent than they were in the preceding horizon xv, and the cerebral hemispheres are relatively smaller than in horizon xvii. It is in the details of special regions, however, that the developmental progress can be most sharply identified, and in this group such distinctive characters are to be found in the nostril, eye, hyoid bar, and especially in the limb buds.

We have seen the primordia of the nostrils make their appearance as two bilaterally symmetrical, discrete, and separate placodes. In the present age group they are still separate and discrete. When they acquired their elevated borders, in earlier specimens, the placodes faced lateralward and their concave floors could be seen in profile views of the embryo. In the younger members of the present age group a part of the floor of the olfactory pit can still be seen in profile views, but in older members, because of the increased relative thickness of the surrounding tissues, the pits face ventralward and their floor can no longer be seen. One sees only the prominent lip which now forms their lateral boundary. In all the specimens of the group the marginal olfactory fold (nasal wing) now overhangs the floor of the depression. It is further to be noted that, in the embryos belonging to the older half of the group, a nasofrontal groove is making its appearance and marks the frontal border of the nose, being exaggerated in shrunken specimens.

The initiation of the formation of pigment in the outer layer of the retina is seen for the first time in horizon xvi. The dark tinge can be seen distinctly in the intact specimen in the older members of the group. It is best seen in the fresh state or in specimens fixed in formalin.

As a consequence of the widening of the head, the two olfactory pits turn ventralward and are brought relatively nearer to each other. Similarly the dorsal segments of the mandibular and hyoid bars are pressed lateralward while their ventral ends are drawn inward and thereby partially disappear from the profile view. On that account these bars and the formation of the face must be studied in decapitated dissections and models. In the present age group the hyoid bar is not bent under toward the median line as markedly as the mandibular bar, and consequently it is more conspicuous than the latter in profile views. It also appears more massive because of the superficial cell proliferations which constitute the precursors of the auricular hillocks of His. In the previous age group it was only in the older borderline embryos that one could recognize the beginning outlines of these characteristic cell masses. In horizon xvi they can be seen on the hyoid bar in every specimen. In horizon xvii there will be more to be said about them. Here it will be merely pointed out that these superficial masses are not to be confused with the primary grooves that subdivide the mandibular and hyoid masses as a whole into dorsal and ventral segments. Except in the younger members of the group, the "third" pharyngeal bar has receded from the surface and is covered over by its more bulky neighbor.

In the preceding age group one could distinguish a rounded hand region as a discrete part of the arm bud and distinct from the arm and shoulder. In horizon xvi the hand region has become differentiated into a central carpal part, around whose edge there projects a thick crescentic flange, which is to form the digital plate. In transparent specimens one can usually see the primary marginal vein coursing along the rim of the latter. The leg bud, though bearing some resemblance to the arm bud, has its own characteristics from the outset. The two are never identical. They appear so at first, but that is only because in their simpler form there are fewer features to be compared, and consequently there are fewer noticeable differences. Closer study, however, reveals their respective individualities. In the present age group three growth centers are revealed in the leg bud, subdividing it into three principal regions. There are: (1) a lateral cephalic part, in which the femoral and obturator nerves of the lumbar plexus terminate; (2) a medial caudal part, in which the peroneal and tibial

nerves of the sacral plexus terminate; and (3) the foot region, to the base of which a part of the tibial nerve already extends. Roughly these represent the thigh, leg, and foot. The pelvis is not yet clearly set off from the thigh. Outline forms of the leg bud as found in horizons xv, xvi, and xvii are shown in figure 1, xvi.

As we come to older horizons and larger embryos, there appears to be an increasing range in variability of size. If, however, we regard size from the standpoint of the percentage variation in the greatest length of the embryo, the size range in these groups is not greatly different from those in earlier horizons. This fact doubtless is to be taken into account in considering both actual size differences and differences resulting from the condition and treatment of the embryos. When the 36 specimens whose external form and inner organization have qualified them as members of horizon xvi are placed in order of their greatest length, they fall into three ranges. There are 7 smaller specimens (19.4 per cent) about 7 mm. long. There are 24 intermediate specimens (66.6 per

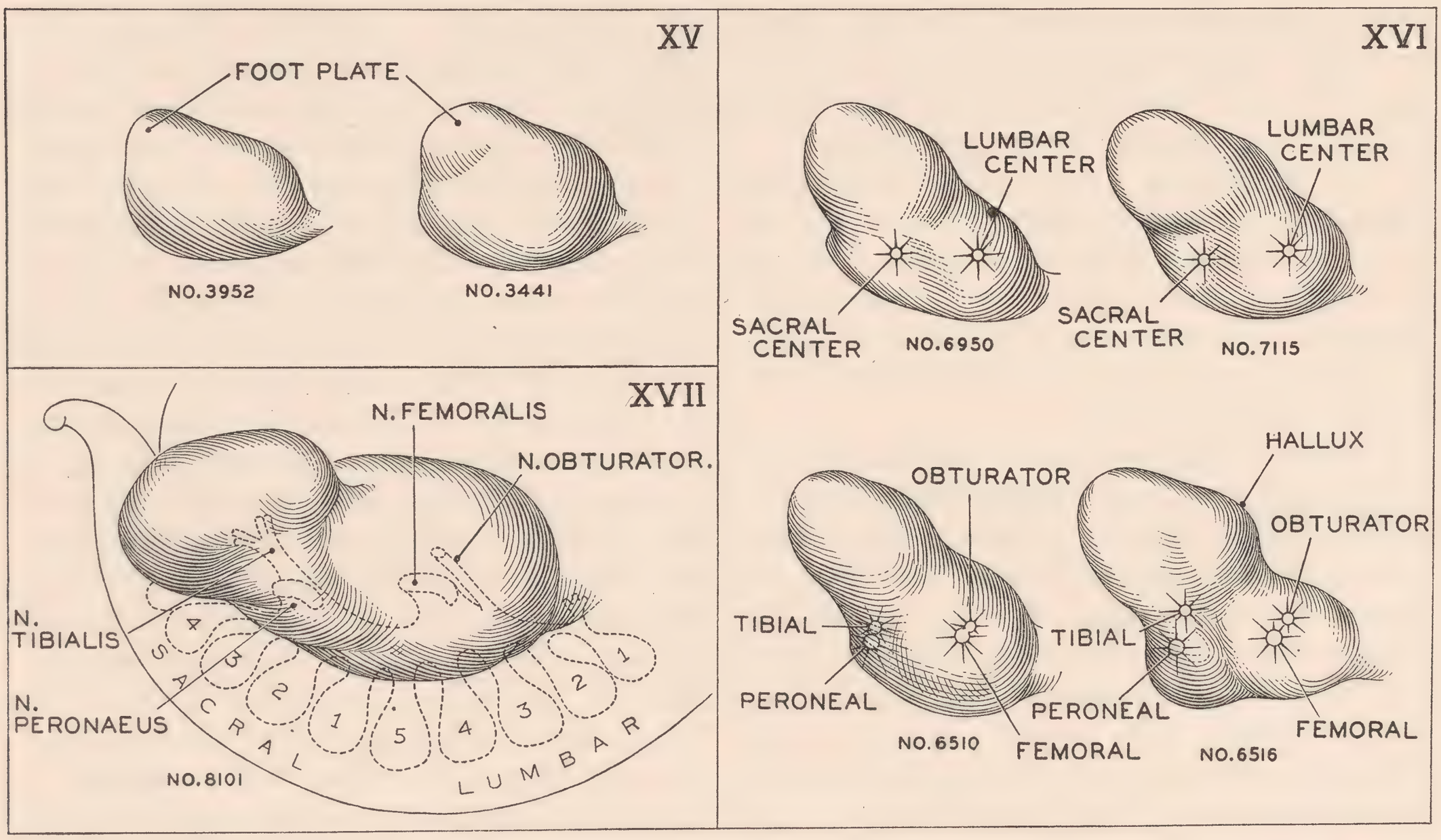



FIG. 1, xvi. Differentiation of the leg bud of horizons xv, xvi, and xvii. In horizon xvii the primary divisions of the leg are clearly indicated by the nerve trunks which branch in a characteristic pattern from the lumbosacral plexus. The femoral and obturator trunks lead to the thigh and, together with the condensation center surrounding them, are responsible for the bulging mass on the proximal and outer part of the limb bud. Coincidently with the breaking apart of this mass into subcenters (muscles), the femoral and obturator trunks split into their respective branches. Each nerve trunk appears to be at its terminus from the beginning. The trunks from the sacral plexus form the central core of the condensation mass of the caudal half of the limb bud; one trunk (N. tibialis) reaches distally to the plantar region of the foot plate. Thus the tibial and peroneal trunks label the primary parts of the limb below the knee. This lumbosacral distribution might be expected from the fact that when the leg-bud condensation is first discernible (horizon xiii, embryos about 4 mm.) its caudal half lies opposite the sacral nerve-ganglion masses, whereas its cranial half similarly lies opposite the lumbar ones. Going backward to horizon xvi, one can still identify the thigh and the more caudal leg by their nerve relation. The foot plate is already determined, and among the older members of the group one can recognize the hallux region. Horizon xv marks the emergence of the foot plate. This horizon is represented only by older members of the group. Among younger members one finds less discrete elevations of the limb bud. ×16.6.

Only in some of the younger members of the group, which are transitional from the preceding age group, can somites and spinal ganglia be seen on the surface throughout the whole length of the trunk. In the older half of horizon xvi, the occipital and cervical somites are obscured by overlying tissue. They can be seen distinctly, however, along that part of the trunk caudal to the arm buds.

cent) having a greatest length of 8 to 11 mm., of which about half are from 9 to 10 mm. long. This is therefore a common length. Finally, there are 5 exceptionally large embryos (14 per cent) ranging from 11.5 to 12.0 mm.

The data on the size of the chorion are even more unsatisfactory than those for horizon xv. Adequate dimensions of the chorion of 17 specimens are found in the records and are listed in the table of embryos below. It will be seen that there is much overlapping in size with those of the preceding age group. There are 4 of them, however, that have a largest diameter of 40 mm. or more, whereas there was only one such in age group xv.

When specimens of this age group are compared with macaque embryos of known ovulation age, they are found to fall midway between embryo no. C 482, 8.6 mm., 32 days, and embryo no. C 479, 11.5 mm., 34 days.[1] Though between these two primates there are already some differences in certain details of external form, one can match them satisfactorily and the matching is further substantiated by their inner structure, as seen in serial sections. On that basis it is concluded that age group xvi of the human embryo corresponds to a stage in the macaque not yet represented in the Carnegie Collection, a stage intermediate to the developmental levels of C 482 and C 479. The ovulation age of the group is therefore estimated to be 33 days ± 1 day.

In brief summary, embryos belonging in horizon xvi are characterized in their external form by nostrils with overhanging borders, and the floor of the olfactory pit in most cases cannot be seen in profile views. The eyes begin to show a dark tinge due to early retinal pigment. Auricular hillocks on the surface of the hyoid bar are becoming distinct. The hand region has become differentiated into a carpus and digital plate. The leg bud exhibits three centers of proliferation representing the thigh, leg, and foot regions. The surface elevations caused by the somites and spinal ganglia have become smoothed over in the cervical region by the overlying embryonic reticular tissue, but beyond the arm they are distinct for the remainder of the trunk. In size, two-thirds of the embryos in this age group range from 8 to 11 mm. in greatest length. The most common length, however, is from 9 to 10 mm. The ovulation age for the group is estimated at 33 ± 1 days.

### Olfactory Pit Region and the Early Skull

The entrance to the mouth of embryos of horizon xvi is still equipped with merely a lower jaw, and even this is in a primitive state of differentiation. The increasing breadth of the brain determines the considerable distance between the two olfactory pits, since they lie ventrolateral to it. The roof of the mouth (stomodeum) slopes forward between the olfactory pits as a broad smooth surface, curving dorsalward over the anterior end of the head, with no interruption to mark the location of an upper jaw. It is still too early to speak in terms of a face. Sketches of this region in two embryos belonging to this age group are shown in figure 2, xvi. The pertinent details should be compared with those of embryos of the succeeding age group shown in figure 1, xvii.

It may be helpful at this point to survey the mesenchyme, which is to play such an important part in the formation of the structures that will comprise the skull and face. It is to be remembered that the mesenchyme of the head is by no means a homogeneous tissue. Instead, it includes cell groups that come from more than one source and that have different potentialities. The group best known consists of the cells that emerge as a sheet or layer from the primitive streak ("middle germ layer") and the related special strand of cells coming from the primitive node (notochord). In this process they make their first step in specialization and correspondingly are no longer so nearly totipotential. From these cells are derived, among other things, the somites and scleromeres (trunk muscles and axial skeleton). Also some of them differentiate into angioblasts and give origin to the blood vascular system. Another important specialization of the mesenchyme results in a coalescence of its larger spaces into channels communicating with the exocoelom, producing an irrigation system, seen at the height of its development in horizon xi. The surface cells of the coelomic system, under the influence of the regions in which they lie, respond by active proliferation and supply the visceral mesenchyme that gives origin to the vascularized muscular and con-

[1] C. H. Heuser and G. L. Streeter, Development of the macaque embryo, Carnegie Inst. Wash. Pub. 525, Contrib. to Embryol., vol. 29, pp. 15–55, see pls. 31, 32, 33, 1941.

nective-tissue coats of the viscera, whereas the residual surface cells become permanent serous membranes and serve as the pericardium, pleura, and peritoneum. These mesenchymal components, of primitive-streak ancestry, can be followed without much difficulty. We can add to them some contributing cells which in the primates appear to detach themselves from the gut endoderm and join in the mesenchymal activities of the gut region. If it is subsequently found that the gut endoderm itself was originally a part of the same germ bed that later becomes the primitive streak, and became detached early, then there is but a temporal difference between these cells and those typically delaminated from the primitive streak.

Especially in the head region, there are other mesenchymal cells that are less well understood than those mentioned in the preceding paragraph. It was chiefly through the morphological studies of Landacre on the amphibian neural crest,[2] and through the results reported by Stone following various experiments in the removal or transplantation of the neural crest, both in salamanders and in frogs,[3, 4] that we learned that a large part of the cartilaginous skull and adjacent loose connective tissue are derivatives of neural ectoderm. In human embryos, where the cytological difference between neural crest cells and the cells of the surrounding mesenchyme is less marked than in the animals studied by Landacre and Stone, the migration and fate of individual crest cells has not been traced with certainty, but until there is evidence to the contrary, it can be assumed that their behavior is the same as in amphibians.

Another characteristic of head mesenchyme is the prevalence of greater thickness of the skin ectoderm over its more condensed areas, suggesting increased functional activity. This is notable in the ventral parts

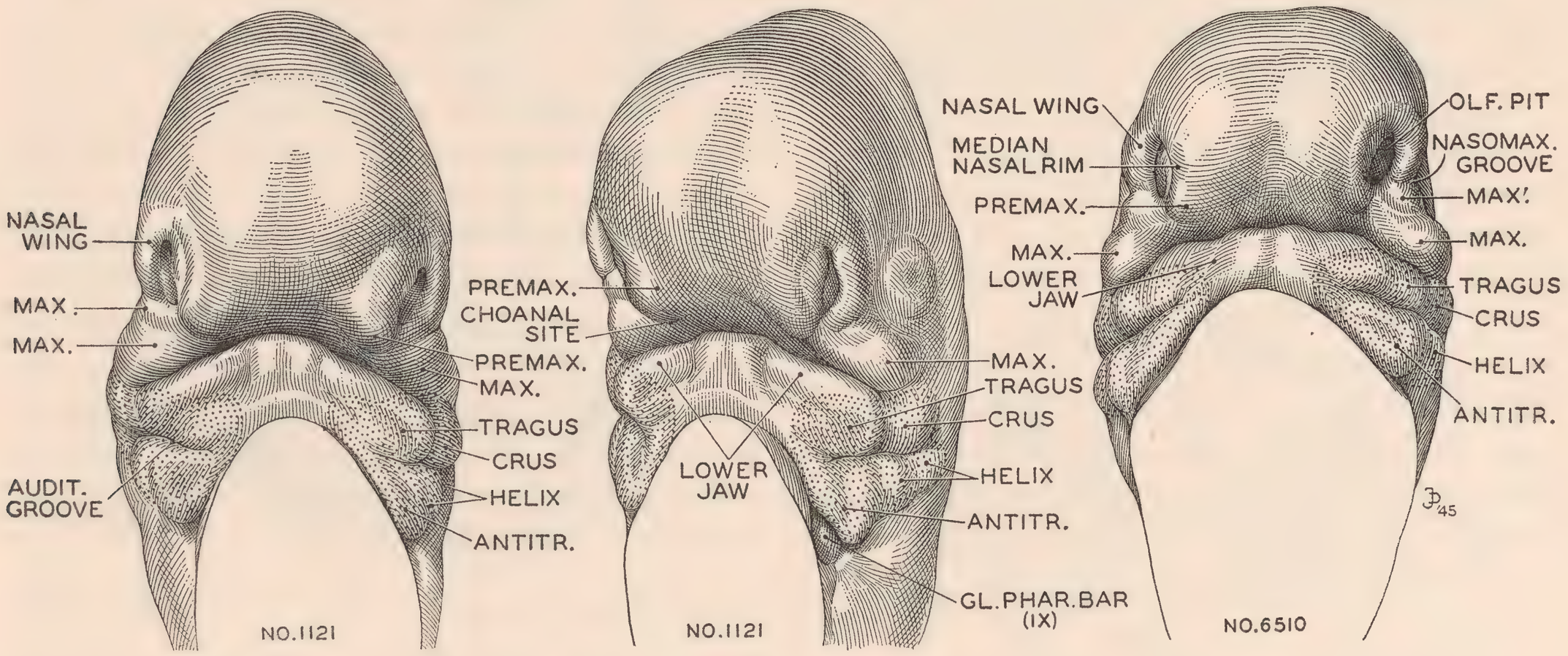



FIG. 2, xvi. Representative views of the olfactory pits and mouth region in horizon xvi. The two views of no. 1121 show a younger member of the group, whereas no. 6510 belongs among the older ones. The right and left olfactory pits show no evidence yet of uniting to form a nose. The interval between them consists of undifferentiated tissue extending from the roof of the stomodeum forward and upward over the frontal region of the head. This is sometimes spoken of as the "frontal process," but if that term is to be applied in horizon xvi one must be satisfied with an idea rather than with a recognizable structure. The growth centers that are to form the upper jaw are widely separated, like the olfactory pits, and are present only as bilaterally placed masses. These include the premaxillary (globular process of His) and maxillary centers, together with a characteristic subdivision of the latter (MAX'.). Though one cannot yet speak of an upper jaw, the lower jaw is definitely marked off and it thus has the distinction of being the first part of the facial equipment to become thus established. The auricular hillocks on the mandibular and hyoid bars are labeled with the parts of the external ear they are to form. Drawings made by J. F. Didusch. Reconstructions made by O. O. Heard. ×12.5.

[2] F. L. Landacre, The fate of the neural crest in the head of urodeles, Jour. Comp. Neurol., vol. 33, pp. 1–43, 1921.

[3] L. S. Stone, Experiments on the development of the cranial ganglia and the lateral line sense organs in *Amblystoma punctatum,* Jour. Exper. Zool., vol. 35, pp. 421–496, 1922.

[4] L. S. Stone, Experiments showing the role of migrating neural crest (mesectoderm) in the formation of head skeleton and loose connective tissue in *Rana palustris,* Arch. f. Entwicklungsmech. d. Org., vol. 118, pp. 40–77, 1929.

of the head, particularly the mandibular and hyoid regions. Such areas stand out in contrast with the loose reticular mesenchyme found over the dorsal parts of the head, where the skin ectoderm is thin and apparently doing nothing more than adapting itself to the expanding size of the underlying brain. Thick skin ectoderm and marked proliferation of the mesenchyme with which it is in contact are likewise characteristic of the limb buds, and doubtless are an expression of some interaction between the two tissues. In embryos at this time the hyoid and the mandibulomaxillary masses, along with their nerve-ganglion trunks, stand out as white opaque conspicuous regions in contrast with the more transparent areas elsewhere, particularly in specimens fixed in formalin. In this they closely resemble the limb buds. Examples are shown in figures 9, 10, 12, plate 1, xvi; figure 23, plate 3, xvi; figure 24, plate 2, xvii; and figures 11 and 14, plate 1, xviii. Their appearance suggests that the limb buds and the group of mesenchymal ridges that form at the sides of the mouth and pharynx around the termination of the large cranial nerves are the product of the same kind of developmental relation between the skin ectoderm and the underlying blastema, plus, of course, whatever the nerves contribute.

As the development of the head progresses, it will be seen that the cranium arises through coalescence and spread of multiple condensation areas (primordia of skull parts) to which skin ectoderm, neural ectoderm, mesenchyme of different origins, and the cranial nerves all contribute materials or act as regulatory factors. These condensations begin bilaterally, and the composite halves can be seen to spread medially to the mid-ventral line, fusing caudalward with the primordium of the axial skeleton and later with the condensed capsules of the right and left otic vesicles. Forward the skull primordia fuse with the condensed areas near the eyes and the olfactory pits. From their initial lateral position the two sides of the cranium gradually spread by interstitial growth and marginal accretion dorsalward, and finally come together over the dorsum of the head. The distinction between cartilaginous and membranous elements of the skull is omitted for the present. It will be taken up when we come to older horizons.

What is important to us now is the status of these structures in horizon xvi. For the greater part, between the thin skin ectoderm and the brain there is found a sparse amount of loose embryonic connective tissue through which passes the network of capillaries and blood vessels supplying the brain wall and cranial nerves. Standing out in contrast there are some fields where the mesenchyme cells by active proliferation have produced condensed areas, or detached foci of growth. They are found in the neighborhood of the eye, and surrounding the otic vesicle and the olfactory plate. To some extent these condensations influence the surface form of the embryo. Thus, around the olfactory pit there is a low elevated ridge, and on the lateral border of the pit, partly owing to the changing shape of the epithelial plate itself and partly to mesenchymal condensation, there is produced a distinct lip ("lateral nasal process"). The condensation in the eye region marks what will become the orbit. The surface form of the embryo head at this time is illustrated by ventral views of typical embryos in figure 2, xvi. These are to be compared with the embryos of the next older stage shown in figure 1, xvii, and with the younger stages shown in figure 1, xv.

In the more ventral parts of the head, mesenchymal condensation exhibits relative precocity. One can recognize a rather dense maxillary field which bulges on the surface as a prominent ridge (see fig. 2, xvi, no. 1121). Under the surface this field blends with the adjacent condensations surrounding the olfactory pit and eye of the same side. The right and left maxillary fields are, however, still widely separated. Between them is the broad region that is to become the roof of the mouth. The only surface markings of this region are two rounded low elevations produced by incipient bilateral condensations constituting the primordia of the primary palate (premaxillary, intermaxillary, or incisive bones). Aside from these modest representatives, there is lacking any upper jaw. In many respects the ventral part of the cranium and the floor of the mouth is more advanced than its sides and top. The nerves are larger, the epithelium is thicker and more specialized in its flangelike extensions, and the mesenchyme is more condensed and shows multiple foci of growth. There is a definite lower jaw, and on the surface of the mandibular and hyoid bars are the condensations of the auricular hillocks, which in a constant manner proceed to form the specific parts of the external ear. These various

things take place before the advent of a definite cartilaginous skull.

In summary, this horizon is characterized by the sinking in of the olfactory pits, due jointly to the form assumed by the olfactory epithelium in its invagination and to the surrounding elevated ridge or lip of condensed mesenchyme which is to become the lateral wing of the nose on that side. Other than a short maxillary ridge and the beginning of the primary palate on each side, there is no upper jaw. The lower jaw is definitely laid down and exhibits multiple centers of condensation. Thus the only representatives of the skull at this time are the above-mentioned separate areas of condensed mesenchyme in addition to the primordium of the axial skeleton which underlies the central nervous system, and projects forward from the trunk into the occipital region.

## Heart

A typical series of sections through the heart of an embryo belonging in this age group is shown in figure 3, xvi. In such sections one can obtain an impression of the relative proportions and orientation of the different elements that make up the total heart. A conspicuous part is taken by the gelatinous reticulum, which thickly invests the primary heart tube everywhere except at the ventricular pouches.[5] The latter consist of two trabeculated recesses opening off

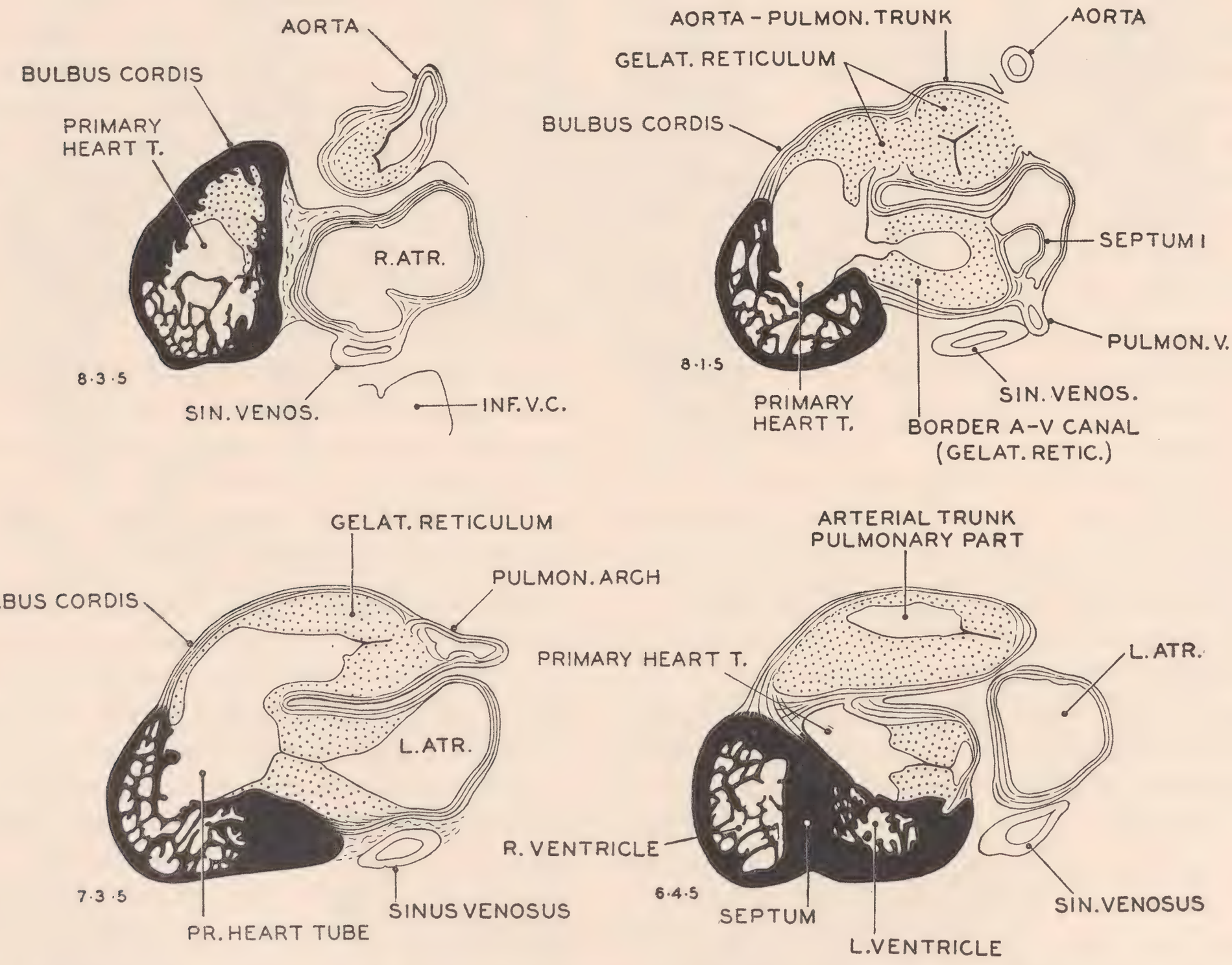



FIG. 3, xvi. Selected sagittal sections through heart of embryo no. 6511, typical of this age group. The primary heart tube, the wall of which is characterized by marked development of gelatinous reticulum, constitutes a conspicuous part of the heart at this time. The ventricles opening off from it are shown in solid black. Section number indicated under each drawing. ×30.

[5] In Tandler's description of the development of the heart (K. von Bardeleben, Handb. d. Anat. d. Menschen, vol. 3, pp. 9–44, see fig. 9, p. 15) is included an excellent picture of the gelatinous reticulum of the primary heart tube as it is seen in the 15-somite embryo (horizon xi). He attached little significance to it; it remained for Davis to demonstrate its nature as a jelly, an important structural element of the living embryo.

from the ventral rim of the heart tube, as has been shown in three dimensions in figure 3, xv. The gelatinous reticulum terminates abruptly on the aorta and pulmonary arch where these become free of the heart. Proximal to this, the endothelium of the two is still found as a common aorta-pulmonary trunk, although the two component regions can be identified. The semilunar valves are not yet present. The site of the tricuspid and mitral valves consists of the gelatinous reticulum which encircles the atrioventricular canal and is usually described as anterior and posterior endocardial cushions. The canal itself takes the form of a widening transverse slit in whose center the endothelium is disappearing, which results in the fusion of the supporting gelatinous reticulum. This will produce two separate channels, the right and left atrioventricular canals.

For the purpose of comparison there is some advantage in placing the different developmental levels of the heart side by side as was done in figure 3, xv. In that figure one can see how the single blood current of the primary heart tube becomes gradually subdivided into two diverse currents, the aortic and pulmonary, without any interruption, during the alterations, in the vital matter of delivery of umbilical and vitelline blood to the systemic vessels. Preceding and perhaps the key to the origin of the two separate currents is a specialization of the primary heart tube in two definite regions along its rim. This is illustrated in figure 3, xv by the embryo representing horizon xi. The primary heart tube is still a single channel from the atrioventricular junction to its outlet at the aortic sac.[6] But in the two separate regions which are to form the right and left ventricles, there is a trabeculated outgrowth of the endothelium as illustrated in the figure. Only endothelium is shown, but it should be added that the overlying myocardium participates in this localized specialization, and in these areas sprouts from the outer myocardial shell extend inward and interdigitate with the endocardial trabeculae. Whether it is the endocardium or the myocardium that initiates this specialization, or whether it is produced jointly, was not determined.

On passing, in the same figure, to an older horizon, namely xiii, the primary heart tube is seen to be more capacious, and considerably longer in the region of the arterial trunk. This increased length includes the part that is usually attributed to the bulbus, a distinction that is not necessarily made for the present purposes. Though the tube is increased in size, there is still little evidence of more than one main blood channel. In horizon xv, however (same figure), the now relatively more voluminous primary heart tube instead of terminating in a common arterial trunk is in the process of separating into aortic and pulmonary channels. The separation is well under way in the oldest stage shown (xvi). One naturally associates the formation of the two separate channels with the growth and activity of the two ventricular pouches, opening from the front margin of the primary heart tube. The ventricular pouches invariably occur at the same sites, and, as one can see in horizon xvi, as they become larger their positions prove favorable for producing directional thrusts or currents toward the pulmonary and aortic outlets, respectively. Consideration of the wide variety of anomalies that occur in these large arteries warns one, however, that ventricular thrusts, if a factor at all, may be only one of many in the development of this our most important organ.

In the reconstructions shown in figure 3, xv it has been noted that the contours are those of the endothelial surfaces, and thus they represent the cavity system. They do not show the gelatinous reticulum which, except in the region of the two ventricular pouches, envelops the primary heart tube throughout its whole length. Surrounding the ventricular pouches, instead of the gelatinous reticulum, there is a thick wall of specialized myocardium, to which reference has already been made. This muscle is quite distinct in its differentiation from the thin myogenic layer that covers the gelatinous reticulum of the primary heart tube and the atrial compartments of the venous part of the heart. The distribution of the two classes of heart muscle is schematically shown in the

[6] The term "primary heart tube" has been generally used to include the sinus venosus and the atria. The writer regards a more restricted use as justified, and makes use of the atrioventricular junction as a boundary line which separates the venous part of the heart from the arterial. These two parts are quite different in their embryological significance, in their structure, and in the manner of their differentiation. By treating the two parts separately one is better able to follow the steps in their organogenesis. The fact that in the more mature heart they in some way become blended can be explained along functional lines and does not appear to the writer as a valid reason for not recognizing their separateness in these earlier horizons.

accompanying figure 4, xvi. Representative sections were taken of embryos of the four successive horizons xiv to xvii. In such sections one can see how the two trabeculated pouches (right and left ventricles) grow the party wall which in horizons xvi and xvii we recognize as the interventricular septum grows from above downward, its crest being its oldest part. Later it will be seen that subsequent coalescence of the crest

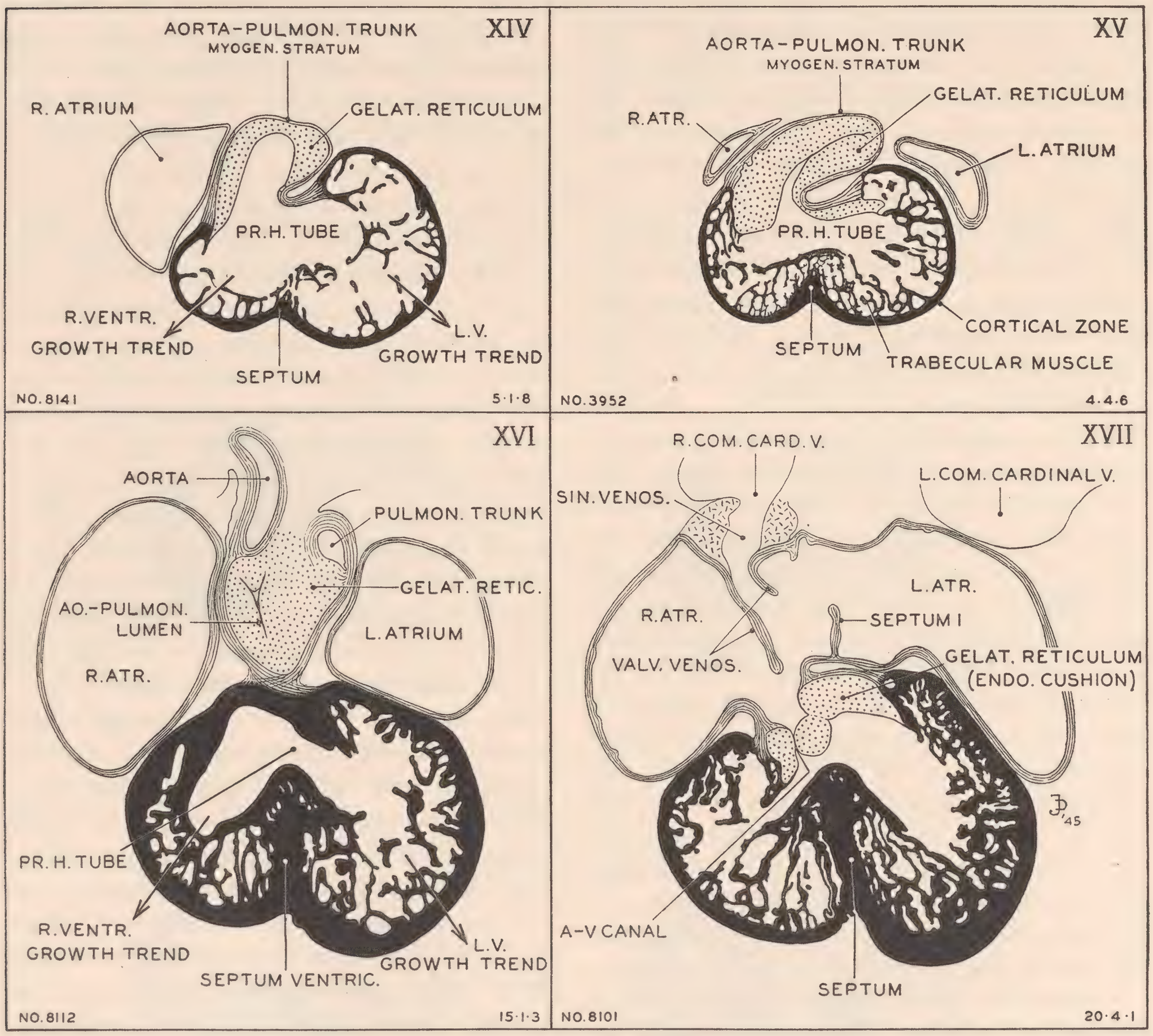



FIG. 4, xvi. Origin of interventricular septum, as seen from horizon xiv to horizon xvii. An example of each horizon is shown. The sections of the first three are taken from embryos cut frontally and the fourth from one cut transversely. This makes them easily comparable. It will be seen that the two ventricles start as separate reticulated pouches, but as they grow outward their myocardial shells coalesce in the formation of a party wall. The crest of the latter is its oldest part, and it is the crest which finally, in horizon xviii, fuses with the tissue of the primary heart tube and produces the membranous septum which separates the cavities of the two ventricles. ×30.

out separately from the primary heart tube. One also sees that when the pouches become larger and deeper they coalesce and acquire a common party wall. The crest of the septum is laid down first and is added to below as the pouches become deeper. In other words, with the fibrous rings from which the tricuspid and mitral valves are to be supported, and the membranous septum, both being late derivatives of the primary heart tube, will close off direct communication between the ventricles over the top of the septal crest.

## Gut Tract

Since the outlines of the tract of gut epithelium for the three successive horizons xv, xvi, and xvii were made with a similar plan and at the same enlargement, the illustrations showing them in figure 5, xv, figure 5, xvi, and figure 6, xvii, respectively, should be compared in detail, from the younger to the older and from the older to the younger; thus one can determine for each of the three the alterations that take place in these intervals of two days' development. One can comprehend from the drawings the changes in relative length, diameters, flexures, and general form of the epithelial tract more quickly and accu-

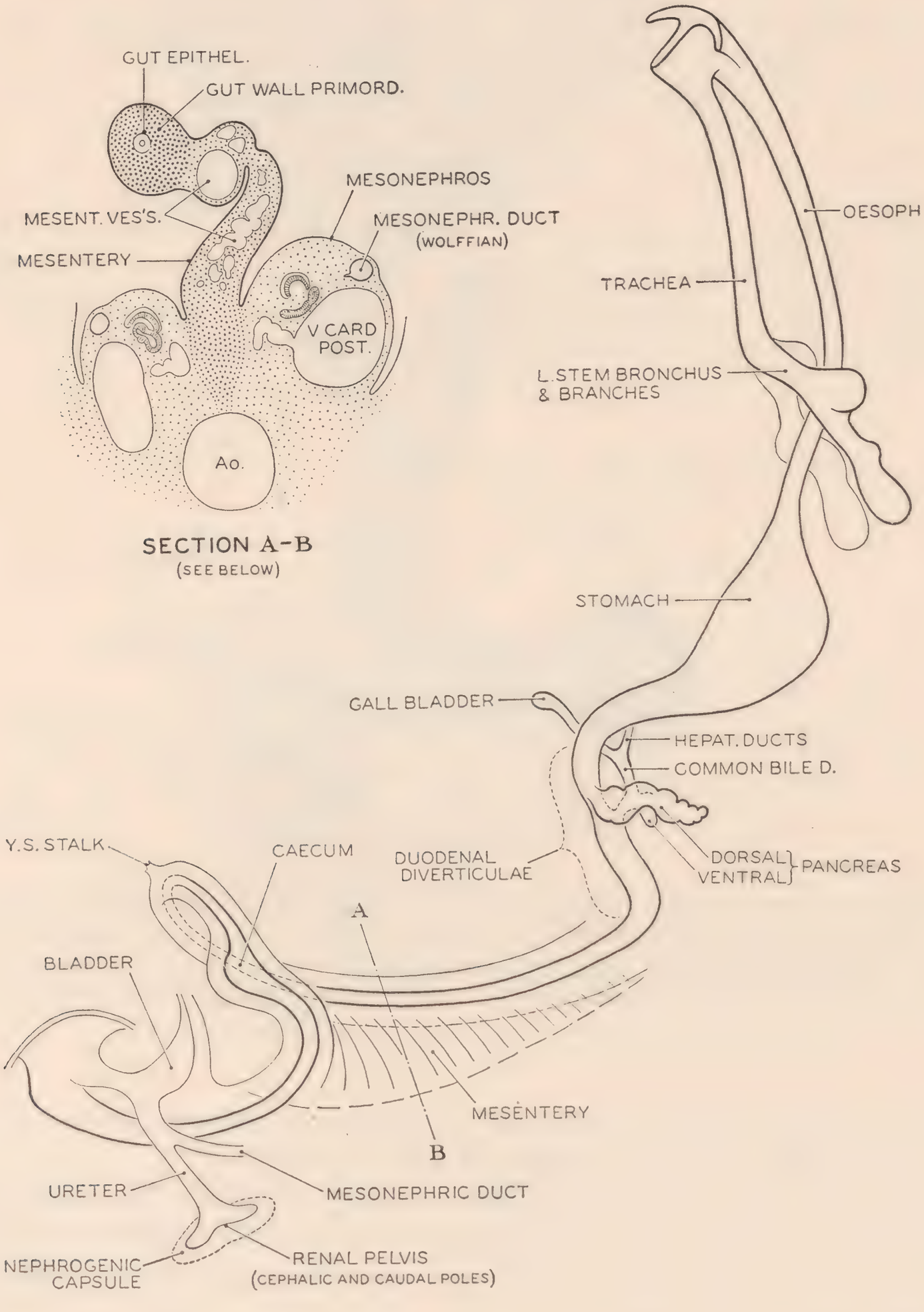



FIG. 5, xvi. Epithelial gut tract of embryo no. 6510, a median member of age group xvi. This sketch is to be compared with the next older and younger horizons, figure 6, xvii and figure 5, xv, shown at the same enlargement. In addition to advancement in details of the separate parts of the tract, it is to be noted that the trachea is now separate from the esophagus up to the level of the larynx. It is also to be noted that there is now a definitive mesentery, the structure and attachment of which is schematically shown in the inset by a section (6-3-3) from embryo no. 1121, of similar age and cut in a favorable plane in the direction shown by line A–B. Drawing made by J. F. Didusch. Reconstruction made by O. O. Heard. ×33.4.

rately than from a detailed word description, and therefore the reader will be spared that.

A striking feature of the gut epithelium is the marked leadership it exhibits in the delineation of the different parts of the tract, and this applies also to such offspring as the lung epithelium, biliary tract, and pancreas. These regions could be identified in the earlier horizons, and now in horizon xvi they stand out in advanced detail (fig. 5, xvi). It is to be particularly noted that we can now speak of a definitive mesentery, beginning near the caudal end of the duodenum and extending downward to include the upper half of the colon. It provides the framework for the primary intestinal loop. The section A–B in this figure shows schematically the structure of this mesentery and its terminus in the primordium of the gut wall. The mesenteric vessels are conspicuous. Here as elsewhere, distinct gut epithelium is surrounded by condensed visceral mesenchyme showing early stages of angiogenesis. The reader should note that a distinction must be made between a definitive mesentery, such as we have at this horizon, and the fanciful or theoretical mesenteries commonly pictured in the textbooks.

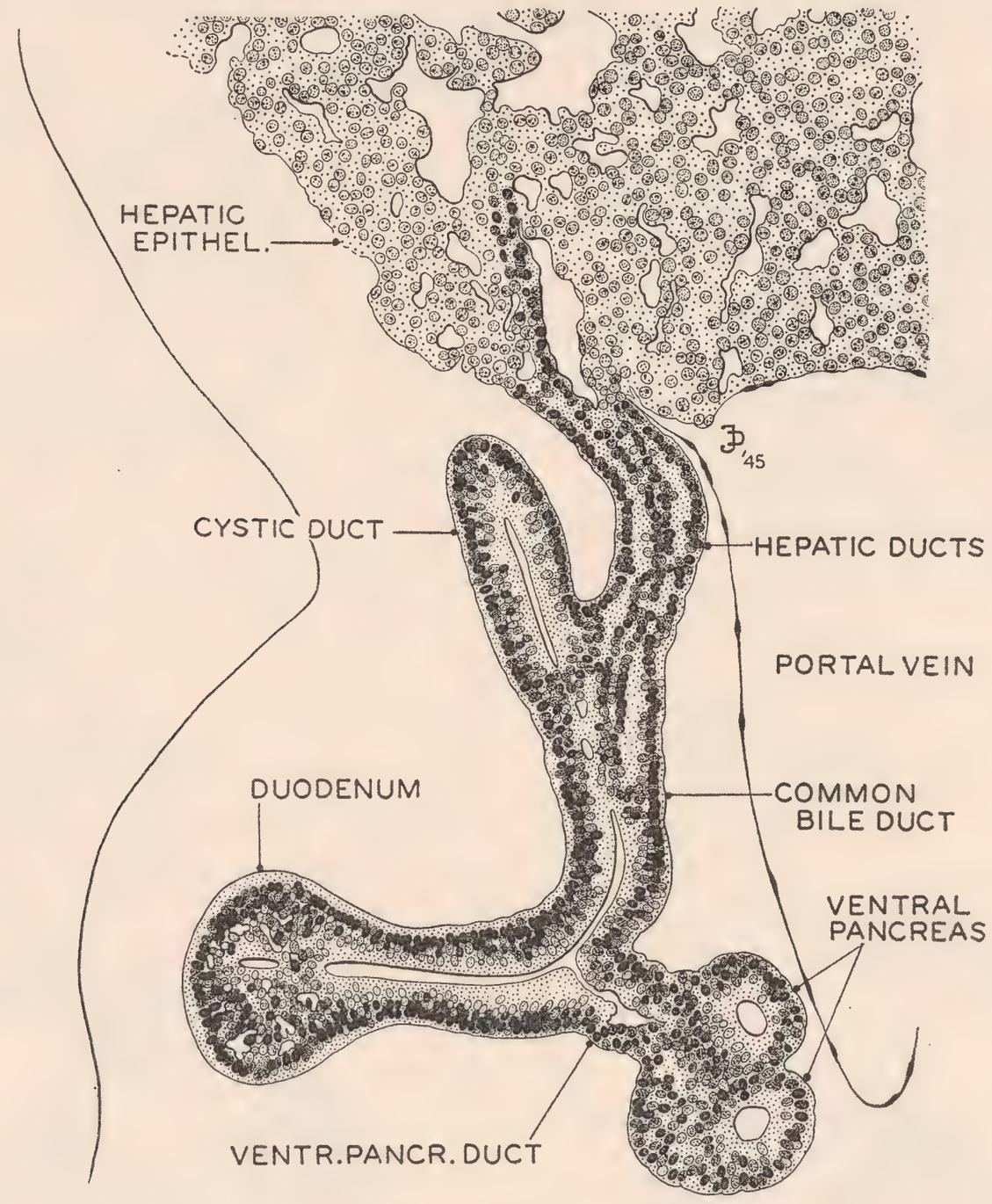



FIG. 6, xvi. Composite drawing of three successive sections showing the termination of the biliary duct system at the duodenum, typical of this age group (embryo no. 6511, sections 8-1-3 to 8-2-1). Only a small part of the cystic duct is shown. The abrupt termination of the hepatic ducts in the hepatic epithelium is characteristic. The biliary duct serves as a stem maintaining the continuity between the hepatic epithelium and the duodenal epithelium, never interrupted from the time of the first outsprouting of the hepatic epithelium. Drawing by J. F. Didusch. ×200.

In the duodenal region the gut epithelium is in the process of active proliferation, and with this there is a noticeable bulging of its contour. Two spindle-form enlargements are thus found, separated by the ducts from the pancreas and the biliary system. A characteristic section through the duodenum at this time is shown in figure 6, xvi. This figure also illustrates the communications of the biliary tract and the differentiation of the duct system. The ventral pancreas still empties by its own duct into the common bile duct

from which it was derived. The duodenal end of the dorsal pancreas is attached to the duodenum in front, and at a level nearer the pylorus than the common bile duct. As can be seen in the figure, the growth of the hepatic ducts produces an intervening stem that isolates the hepatic epithelium from the lower biliary system. Examination of the extreme distal tip of the hepatic ducts, possible in the section illustrated, indicates that it is a differentiation *in situ* of the duct epithelium; that is, a conversion and proliferation of cells already present rather than a penetration by foreign cells. The section shown is a very early stage in the process. There are no discernible hepatic ducts at this time in any other part of the large liver mass.

The lung epithelium, which can now be spoken of as a bronchial tree, is shown in figure 8, xvi. The right and left stem bronchi exhibit characteristic asymmetries. They each end in a bulbous terminal growth center, and along the sides give off a characteristic type of elongating lateral branches. In the figure, three examples of development are shown, to cover the range that exists in this age group.

## Central Nervous System

The brain of horizon xvi, now two days older, has increased about one-fourth or one-fifth in its principal dimensions. At the same time it has acquired further differentiation in form. The details in this transformation can be seen by comparing figure 7, xvi with figure 6, xv. Among the items to be noted is the increase in size of the collicular plate and the consequent relatively deeper groove caudal to it, defining the isthmus more sharply. The tegmental plate is extending caudalward, crowding the rhombic grooves to the side, and it is about to coalesce with the basal plate. As this occurs, the two mid-line longitudinal depressions opposite the trigeminal and facial grooves, heretofore so constant, become thicker and tend to disappear. The pretectal field is marked by the fiber bundles arising from it and crossing in the posterior commissure. The outlines of the thalamus are sharper. The development of a marginal ridge along the anterior border of the thalamus defines it more clearly. The ridge separates it from the subthalamic region, where the character of brain wall differs markedly from that in the thalamic zone. The

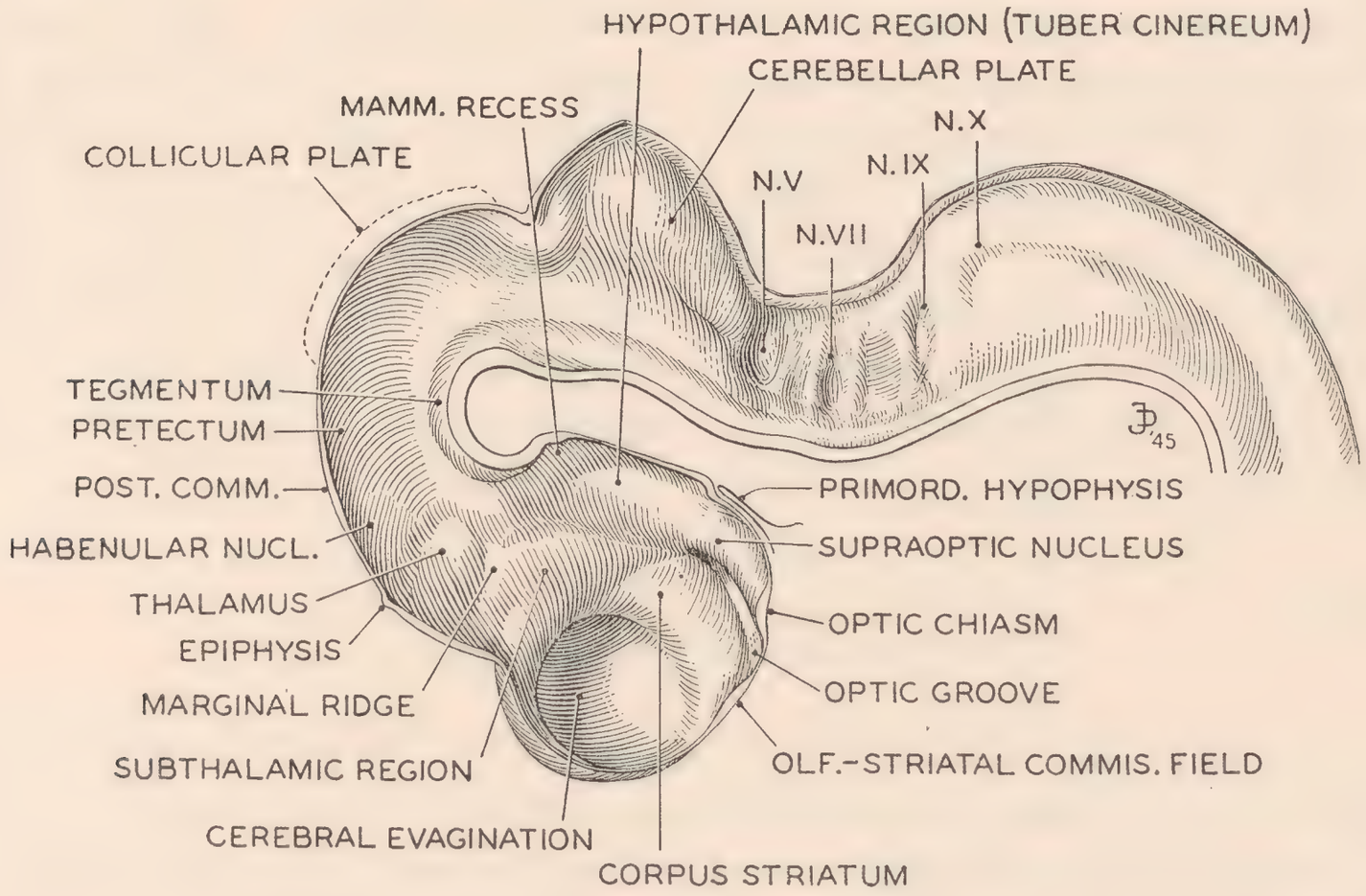



Fig. 7, xvi. Reconstruction of right half of brain of embryo no. 6510, typical of age group xvi. A marginal ridge now begins to mark off the thalamus from the subthalamic region, lying rostral and below it. The tegmentum is extending caudalward to become continuous with the basal plate of the hypoglossal region. A slight evagination of the infundibular floor in the mid-line marks the beginning of the neural lobe of the hypophysis. This figure is to be compared with the others of the brain series (fig. 6, xv; fig. 10, xvii; and fig. 8, xviii), all being of the same magnification. Drawing made by J. F. Didusch. Reconstruction made by O. O. Heard. ×12.5.

thin strip of brain wall composing the optic groove leads laterally directly into the optic evagination, now reduced to a narrow slit. As a final item, it is in this age group that the evagination of the floor begins in the region of the hypophysis, and that will form the neural hypophysis.

### *Eye and Ear*

It is at this point that definitive pigment makes its appearance in the outer coat of the retina, and there is thus provided a useful character for marking the boundary line between horizons xv and xvi. Whether pigment is present or absent is decided by the arbitrary rule that if the pigment granules can be seen clearly with an 8-mm. objective, then pigment is recorded as present. Under greater magnification fine refractive granules that are differentiating into pigment can be seen earlier than this. Such specimens are found among the older members of age group xv. Photographs of the eye and its environment as it is seen in horizon xvi are shown in figures 29 and 30 on plate 3, xvii. Here the lens vesicle has become free from the overlying skin epithelium. A sharply marked-off segment at the base of the lens vesicle exhibits the beginning of lens fiber differentiation. This primordium of the body of the lens is characterized by a basal nuclear layer and a central clear area that consists of the elongating cell bodies. The remainder of the wall of the lens vesicle is left to form the expanded epithelial covering of the front of the lens in the later stages, when the lens cavity has been reduced to a slit by the encroachment of the enlarging lens body. In the relatively large lens cavity of the group we are now studying there are found small clusters of disintegrating cells, being the remnants of what have been tentatively designated as unwanted elements extruded by the lens plate epithelium during its invagination. These remnants are still found in later stages.

One can see the retinal pigment in the photographs even at this low magnification. When the same specimens are photographed under higher magnification, additional details are revealed, as is shown in figure 28, plate 3, xviii. These will be referred to again when we come to the older age groups.

The general characteristics of the ear vesicle of horizon xvi are shown in the longitudinal and transverse sections (embryos no. 422 and no. 6510) illustrated in figure 8, xvi. The relatively long endolymphatic appendage is a prominent character. The remainder of the vesicle is elongating so that one can distinguish the primordium of the cochlear pouch, though it is not definitely marked off from the vestibular region of which it is an extension. The semicircular ducts are represented only as thickened rims of the triangular part of the vestibular pouch.

## Summary of Horizon XVI

Aside from the characters of external form which were described under that heading, there are other more informative changes which take place in the inner structure of this group of specimens and which are found only on serial section. A group of such characters are collected in figure 8, xvi, to emphasize their syndromic occurrence as compared with the same structures in older and younger horizons for which similar illustrations will be found (fig. 7, xv; fig. 11, xvii; and fig. 10, xviii).

Among the structural characters most useful in determining whether or not a given embryo belongs in this particular developmental horizon, the following are to be mentioned. Definitive pigment granules are present in the outer coat of the retina. The posterior lobe of the hypophysis is beginning to evaginate. In the heart, the central part of the wide atrioventricular canal exhibits the process of fusion of the opposing surfaces of the endothelium and the surrounding gelatinous reticulum, producing right and left canals. An epithelial bronchial tree is present, consisting of stem bronchi with definite elongating primary branches. A definitive abdominal gut mesentery is present, extending from the lower end of the duodenum to the upper part of the colon. There is an elongated ureter expanding into a renal pelvis which in the older members is differentiated into upper and lower poles. If examination of a sectioned embryo reveals that it has all these characters, one can be sure that it belongs in horizon xvi.

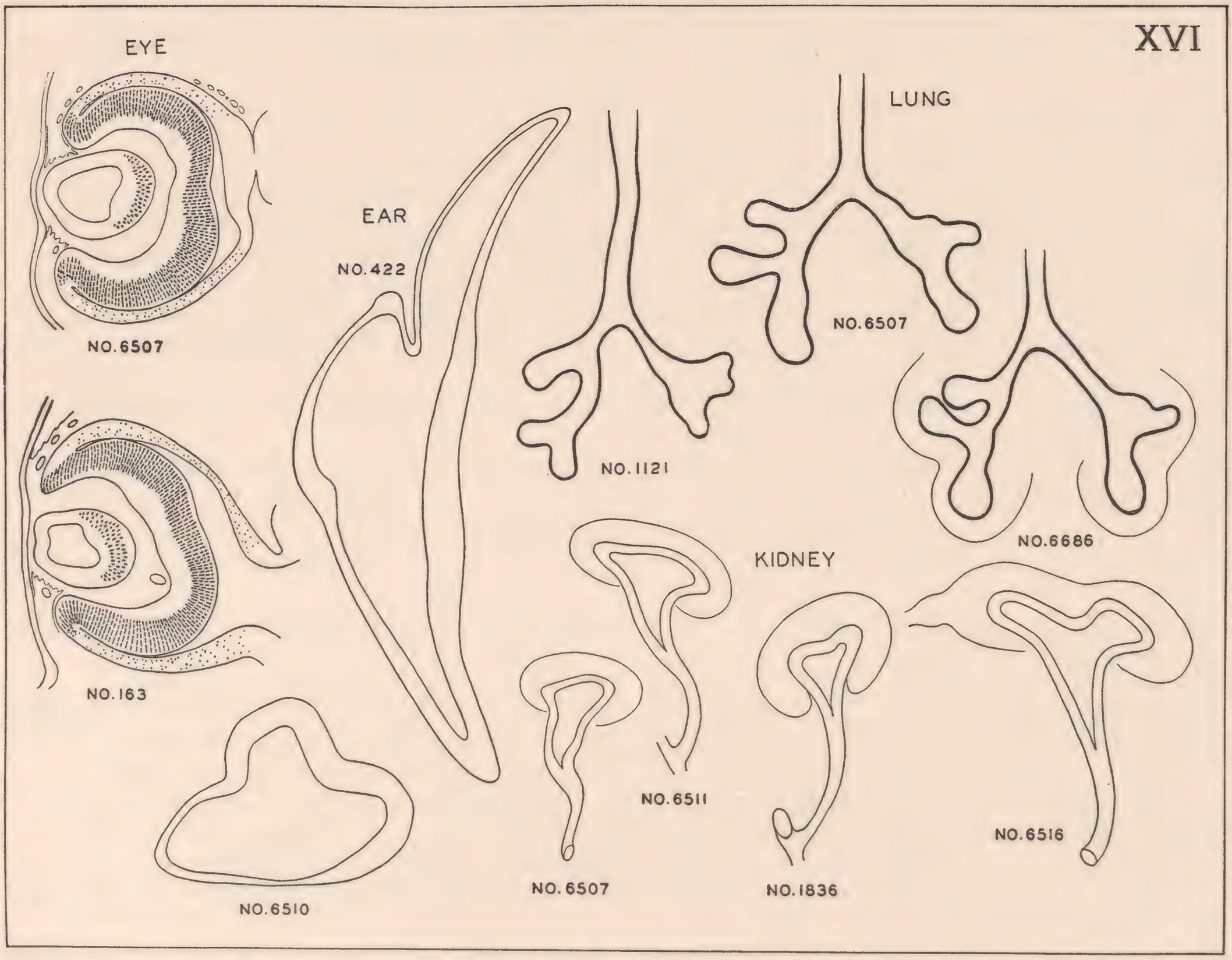



FIG. 8, xvi. Syndrome of characters exhibited by each of the embryos listed under age group xvi. In the eye the outer layer of the retina has pigment visible under medium magnification. The ear vesicle exhibits thinning of the wall that will later be absorbed, but the latter has not yet begun to bend inward. The stem bronchi have definite elongating branches. The ureter is elongated and its pelvic expansion has an upper and lower pole. It will be seen that in these characters the embryo of this horizon differs definitely from those of the preceding and following horizons. Bronchial trees $\times 40$; other organs $\times 80$.

## Normal Embryos in Carnegie Collection Belonging in Horizon XVI

Following the same plan utilized in former age groups, there are herewith listed the embryos available for this study. The explanatory notes supplied under age group xiii apply also here. It is to be noted that in embryos marked with an asterisk (*), the length given is an adjusted one, an estimated proper allowance having been made for unusual fixation or handling.

### List of Embryos

| Serial no. | Size (mm.) | Grade | Fixation | Cutting medium | Plane | Thinness (microns) | Stain | Date, donor, and remarks |
|---|---|---|---|---|---|---|---|---|
| 163.. | E., 9.0<br>Ov., 35 × 35 × 20 | Good | Formol | P | Trans. | 20 | Al. coch. | 1899, T. C. Smith, Washington, D. C.<br>Used by Drs. Bardeen and Lewis |
| 221.. | E., 7.5<br>Ov., 40 × 33 × 33 | Poor | Formol | P | Sag. | 20 | Al. coch. | 1903, R. G. Perkins, Cleveland, Ohio<br>Considerable maceration |
| 383.. | E., 7.0<br>Ov., 15 × 15 × 15 | Poor | Formol | P | Trans. | 10 | Al. carm. H. and C.-R. | 1904, R. B. Normant (Brödel), Baltimore<br>No record of external form |
| 397.. | E., 8.0<br>Ov., 15 × 15 × 15 | Poor | Formol | P | Trans. | 10 | H.-E. | 1907, H. B. Athey (Brödel), Baltimore |
| 422.. | E., 9.0<br>Ov., 30 × 30 × 30 | Poor | Alc. | P | Trans. | 40 | Al. coch. | 1910, G. L. Hunner (Brödel), Baltimore<br>Tubal specimen; some maceration |
| 617.. | E., 7.0<br>Ov., 18 × 14 × 12 | Good | Formol | P | Trans. | 15 | Al. coch. | 1912, R. F. Rand, New Haven, Conn.<br>Median in group; some tissues excellent |
| 792.. | E., 8.0<br>Ov., 40 × 30 × 30 | Good | Formol | P | Trans. | 20 | Al. coch. | 1913, G. C. McCormick, Sparrows Pt., Md.<br>Old in group |
| 887.. | E., 9.0<br>Ov., 31 × 28 × 17 | Good | Formol | P | Trans. | 40 | Al. coch. | 1914, J. H. Groshans, Baltimore<br>Near next older group; circa 34 somites |
| 1121.. | E., 11.8<br>Ov., ..... | Good | Corros. acetic | P | Frontal | 40 | Al. coch. | 1915, D. B. Casler, Baltimore<br>Operative; median in group |
| 1197.. | E., 10.0<br>Ov., 23 × 19 × 15 | Good | Formol | C | Sag. | 20 | H.-E. or. G. | 1915, H. Jeidell, Salt Lake City, Utah<br>Old in group |
| 1544.. | E., 7.2<br>Ov., ..... | Good | Zenker | P | Sag. | 20 | Al. coch. | 1916, W. D. Fleming, Denver, Colo.<br>Tubal specimen; some mechanical injury |
| 1836.. | E., 11.0<br>Ov., ..... | Good | Formol | P | Trans. | 20 | H.-E. | 1917, W. Wilson, Memphis, Tex.<br>In oldest third of group |
| 4677.. | E., 9.5<br>Ov., 48 × 36 × 30 | Good | Formol | P | Trans. | 10 | Al. coch. | 1924, G. C. McCormick, Sparrows Pt., Md.<br>Median place in group |
| 5515.. | E., 12.0<br>Ov., 47 × 37 × 25 | Good | Formol | C-P | Trans. | 10 | H.-E. | 1927, R. G. Craig, Baltimore<br>Near transition to next older group |
| 6054.. | E., 7.0<br>Ov., 21 × 17 × 12 | Good | Formol | C-P | Trans. | 8 | H.-E. | 1930, V. N. Norwood, Baltimore<br>Among youngest third |
| 6507.. | E., 9.0*<br>Ov., ..... | Exc. | Corros. acetic | C-P | Frontal | 8 | Al. coch. | Transition, median to oldest third of group; first four slides 10-micron sections |
| 6509.. | E., 8.1*<br>Ov., ..... | Exc. | Corros. acetic | C-P | Frontal | 10 | Al. coch. | Transition, youngest to median third of group; histology particularly good |
| 6510.. | E., 10.1*<br>Ov., ..... | Exc. | Corros. acetic | C-P | Frontal | 10 | Al. coch. | About same stage as no. 6507 |

(*Continued on following page*)

LIST OF EMBRYOS—*Continued*

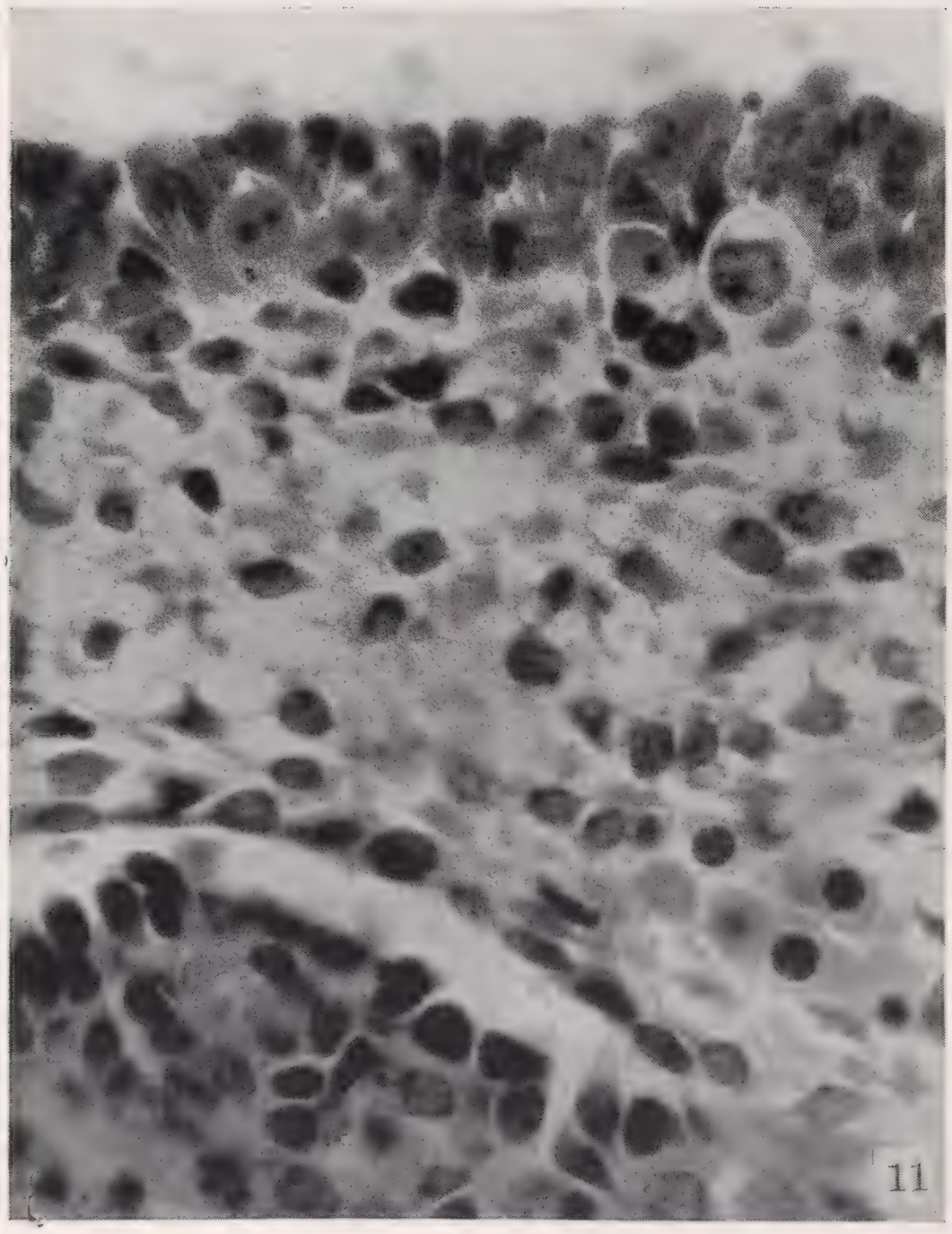
| Serial no. | Size (mm.) | Grade | Fixation | Cutting medium | Plane | Thinness (microns) | Stain | Date, donor, and remarks |
|---|---|---|---|---|---|---|---|---|
| 6511.. | E., 8.1*<br>Ov., ..... | Good | Corros. acetic | C-P | Sag. | 10 | Al. coch. iron H. | In oldest third of group; surface of embryo injured by fixative |
| 6512.. | E., 7.0*<br>Ov., ..... | Exc. | Corros. acetic | C-P | Trans. | 10 | Al. coch. | Young; near transition from preceding group |
| 6513.. | E., 7.2*<br>Ov., ..... | Good | Corros. acetic | C-P | Frontal | 10 | Al. coch. | Youngest specimen of group; surface of embryo gelatinized by fixative |
| 6514.. | E., 9.0*<br>Ov., ..... | Poor | Corros. acetic | C-P | Sag. | 10 | Al. coch. | In oldest third of group; marked fragmentation in cutting |
| 6516.. | E., 10.5*<br>Ov., ..... | Good | Corros. acetic | C-P | Sag. | 8 | Al. coch. | In oldest third of group; histology excellent; double ureter and kidney on left side |
| 6517.. | E., 10.5*<br>Ov., ..... | Exc. | Corros. acetic | C-P | Trans. | 8 | Al. coch. | About same degree of development as no. 6516 |
| 6686.. | E., 11.0<br>Ov., 17 × 17 × 17 | Poor | Formol | C-P | Frontal | 20 | Al. coch. | 1933, F. A. Hemsath, New York<br>Tubal specimen; some maceration |
| 6750.. | E., 10.0<br>Ov., ..... | Good | Formol | C-P | Trans. | 10 | H. and phloxine | 1933, H. Van Horn, Harrisburg, Penna.<br>Near older border line; tubal specimen |
| 6909.. | E., 11.0<br>Ov., ..... | Good | Bouin | C-P | Frontal | 10 | H.-E. | 1934, J. L. McKelvey, Baltimore<br>Tubal specimen; near older border line |
| 6931.. | E., 8.8<br>Ov., 37 × 33 × 16 | Good | Formol | C-P | Frontal | 10 | Al. coch. phloxine | 1934, Karl Wilson, Rochester, N. Y.<br>Among youngest third of group; type specimen |
| 6950.. | E., 9.0<br>Ov., 31 × 20 × 18 | Good | Formol | C-P | Trans. | 10 | H.-E. | 1934, F. A. Hemsath, Jersey City, N. J.<br>Tubal specimen; some fragmentation of tissues |
| 7115.. | E., 9.7<br>Ov., 30 × 23 × 15 | Exc. | Bouin | C-P | Frontal | 10 | H. and phloxine | 1935, A. F. Guttmacher, Baltimore<br>Operative specimen; near younger border line |
| 7629.. | E., 11.5<br>Ov., 31 × 31 | Good | Formol | C-P | Frontal | 10 | Al. coch. phloxine | 1939, L. Wharton, Baltimore<br>Supravaginal hysterectomy; oldest specimen in group |
| 7804.. | E., 9.5<br>Ov., 26 × 21 × 16 | Good | Formol | C-P | Trans. | 10 | H.-E. | 1940, A. Plaut, New York<br>In youngest third of group |
| 7897.. | E., 12.2<br>Ov., 31 × 24 × 23 | Good | Formol | C-P | Trans. | 10 | H.-E. | 1941, W. D. Noble, Easton, Md.<br>Tubal specimen; near older border line of group |
| 8098.. | E., 10.0<br>Ov., 30 | Good | Formol | C-P | Frontal | 6 | H.-E. | 1942, A. Plaut, New York<br>Tubal specimen; median in group |
| 8112.. | E., 10.9<br>Ov., ..... | Exc. | Bouin | C-P | Frontal | 8 | H.-E. | 1943, E. H. Richardson, Baltimore<br>In oldest third of group |
| 8179.. | E., 11.9<br>Ov., 23 × 18 × 17 | Good | Formol | C-P | Frontal | 10 | H.-E. | 1943, A. Plaut, New York<br>Tubal specimen; histology excellent |

FIGS. 9, 10, 12. Three views showing particularly well the characteristics of a formalin-fixed specimen typical of the younger half of age group xvi (embryo no. 8098). The limb buds are thicker than in the older members of the preceding age group, and the marginal vein is now distinct in the leg bud. All views ×8.

FIG. 11. The gonad region of the same embryo (section 10-5-4), showing proliferation and beginning invasion of the coelomic epithelium. It also shows the early presence of primary germ cells, three of which can be seen. The coelomic cells tend immediately to cluster around them. The larger part of the picture consists of the reticulum of the mesonephros, with early angioblasts, and a part of a glomerulus. ×800.

9

10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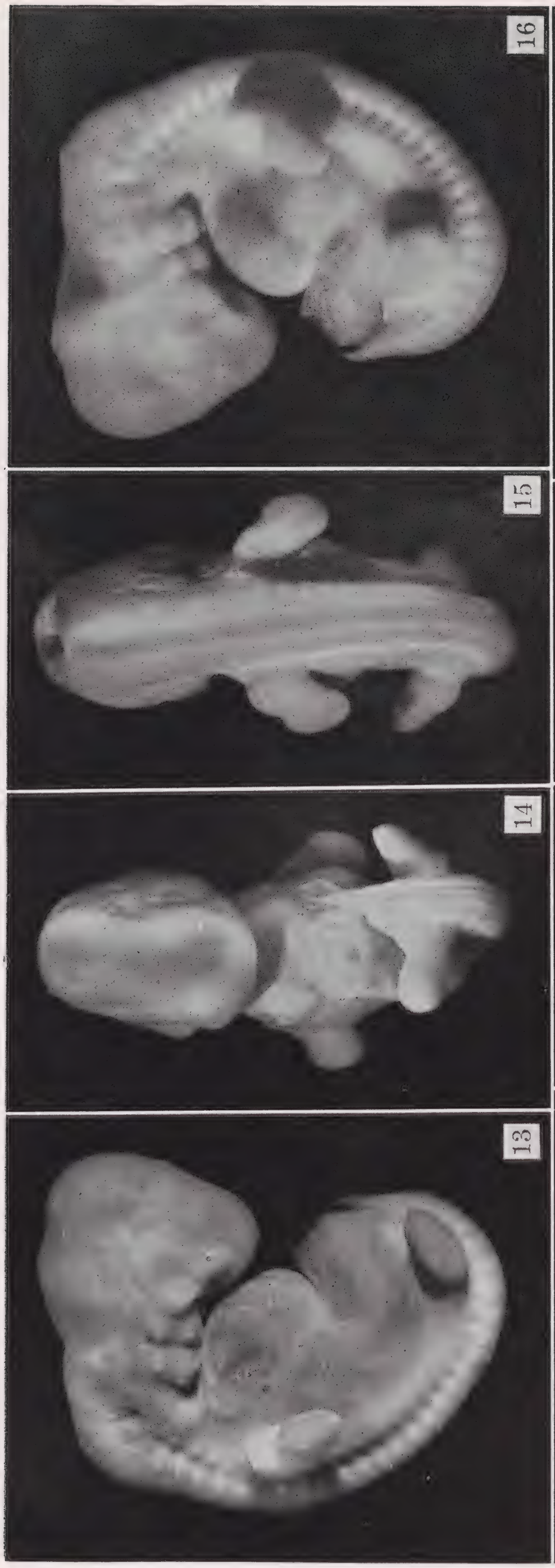
12

Two embryos, one of the youngest (no. 6054) and one of the oldest (no. 8112) members of age group xvi. The difference between them is exaggerated by the fact that the younger specimen was fixed in formalin and then shrunken. It illustrates the uncertainty of depending entirely on external form for alignment of embryos in their developmental order. Both of these qualify for this age group on the basis of their internal structure.

Figures 13 and 15 show the shrinkage and sinking in of the roof of the fourth ventricle. The distinctness of the ganglion-somite masses is also an effect of the shrinkage. The auricular hillocks and the limb buds are found to provide the most reliable of the surface characters for this period of development.

Figs. 13–16. No. 6054, ×10.

Figs. 17–20. No. 8112, ×7.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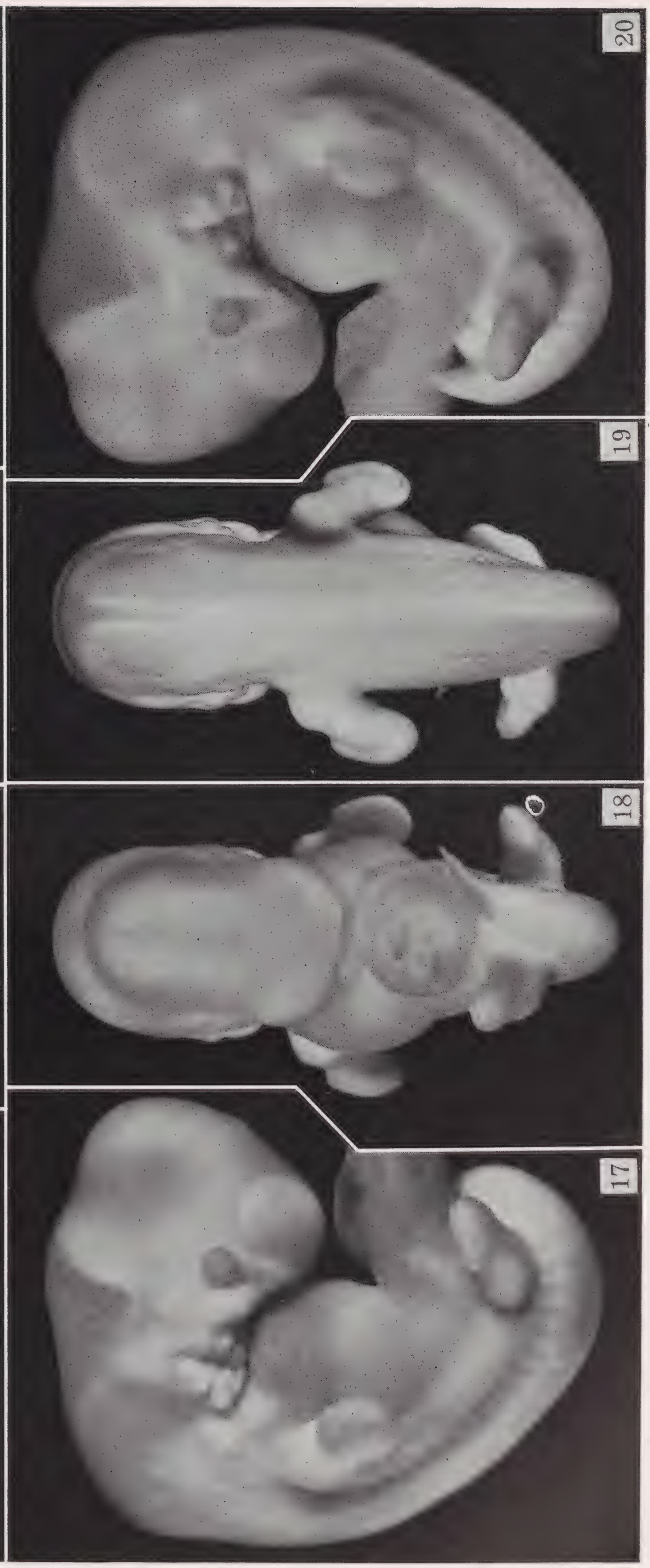
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## Plate 3, xvi

All the embryos shown on this plate have been cut in serial sections, and in their microscopic structure qualify as members of age group xvi. The differences between them are for the most part accounted for by the factors of fixation, shrinkage, manner of illumination, and original condition of the specimen. The ear vesicle, where it can be seen, the auricular hillocks, the lateral nasal wing, the disappearing third pharyngeal bar, the distinct hand plate, and the rounded foot plate with its early marginal vein are characteristics common to all these specimens.

The numbers and enlargement of the embryos are as follows:

Fig. 21. No. 6750, ×6.
Fig. 22. No. 7115, ×6.
Fig. 23. No. 7804, ×6.
Fig. 24. No. 6517, ×6.
Fig. 25. No. 6511, ×6.
Fig. 26. No. 6510, ×6.
Fig. 27. No. 6514, ×6.
Fig. 28. No. 8179, ×5.
Fig. 29. No. 7629, ×5.

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## HORIZON XVII

### External Form, Size, and Age

As a result of the precocious growth of the brain at this time, the head of the embryo appears relatively large. The head, together with that part of the neck lying above the extended ventral line of the face, which includes the upper four cervical segments, now has about the same profile area as the remainder of the body. This is a greater proportion than existed in any previous horizon. There is also a noticeable increase in the fronto-midbrain length of the head, measured from the most frontal point of the forehead to the caudal border of the acoustic colliculus, just in front of the precerebellar notch (isthmus). Furthermore, both the head and the thoracic part of the body have become wider, whereas the lumbosacral region remains more slender. Up to this time, under normal conditions, embryos have had a curved dorsal contour interrupted only by the cervical flexure. The trunk in horizon xvii becomes straighter and therewith begins to acquire a slight lumbar flexure, the causative factors for which are to be found in the transformations of the inner structures, as later we shall see. The caudal termination of the trunk, instead of tapering smoothly as heretofore, now begins to have an abrupt recessive character which will result in the caudal filament of later stages.

So much for the general body form. Examining special parts more closely, one sees in the olfactory region in profiles of all specimens of the group a distinct nasofrontal groove. The olfactory pit opens ventralward and cannot be seen in full profile views. To examine the nostril region it is necessary to have decapitated specimens or models. Further details of the olfactory region will be returned to in a special section below, dealing with the formation of the face.

It is in this age group that the auricular hillocks of His [1] exhibit their characteristic form. They are shown in figure 2, xvi, also in figure 1, xvii. They consist of six circumscribed superficial condensations, three (nos. 1 to 3) on the mandibular bar and three (nos. 4 to 6) on the hyoid bar. The latter are more prominent and are destined to form the pinna of the ear. The three on the caudal surface of the mandibular bar are less sharply outlined. The lowest of them becomes the tragus, and the upper two join in the formation of the crus helicis. Between these two rows of hillocks the hyomandibular groove increases in width and depth, thereby initiating the formation of the concha and the meatus acusticus externus. It is common for the hillocks to coalesce at this time and take the form of a key plate. The opening for the key is encroached upon by the second and fifth hillocks, and the key slot is thus separated into upper and lower parts, like the numeral 8.

The transformation of this region into the external ear has been described at some length in an earlier paper [2] and need not detain us here. It is to be noted, however, that only minimal and superficial parts of the mandibular and hyoid bars participate in the building of the external ear. The rigid control in the organization of this specialized skin structure appears to reside in the surface epithelium. This explains the fact that although there is great individual variation in adult ears, they exhibit a striking bilateral symmetry in any given person. It is an expression of the high degree of their genetic substratum. In spite, however, of their apparent independence of other structures, their development still keeps in step with that of other organs, and one can fairly well determine, from the status of the external ear, the horizon to which a given embryo belongs.

The arm bud of horizon xvii is distinguished from that of horizon xvi by the acquisition of finger rays. They are only slightly indicated in the young borderline members of the group, but are clearly demarcated in all others. The hand plate of the oldest members begins to have a crenated rim due to the projecting tips of the individual digits. This crenation becomes a prominent characteristic of the next older age group.

An outline of the leg bud, as found in horizon xvii, is shown in figure 1, xvi, where it is compared with the leg bud of earlier age groups, all being drawn at the same magnification. It will be noted that besides

[1] W. His, Die Formentwickelung des äusseren Ohres, Anatomie menschlicher Embryonen, pt. 3, pp. 211–221, Leipzig, 1885.

[2] G. L. Streeter, Development of the auricle in the human embryo, Carnegie Inst. Wash. Pub. 277, Contrib. to Embryol., vol. 14, pp. 111–138, 1922.

having increased in length and general mass, it now has a rounded digital plate, set off from the tarsal region and leg. One can also begin to see, at the junction of the leg bud and trunk, evidence of more slowly developing tissue that will eventually become the muscles and bones of the pelvic girdle.

Turning to the somites, it is found that these do not influence the surface markings except in the lumbar and sacral regions, where they stand out as definite elevations. In younger members of the group they can be seen as high up as the mid-thoracic levels. Higher than this, throughout the cervical and upper thoracic regions, the increase in superficial embryonic connective tissue obscures the somites and spinal ganglia. When embryos are abnormally transparent the somites can be seen even here.

The size of the 25 embryos falling in this age group ranges in greatest length from 10.0 to 14.5 mm. This does not include one which had a deformed head, making it unsuitable for size data. If we set aside the 5 largest specimens, having a length of 14.0 to 14.5 mm., and the 3 smallest ones, having a length of 10.0 to 10.8 mm., there remain 17 embryos (68 per cent) ranging from 11.0 to 13.6 mm. in length. Embryos of this horizon are, therefore, most likely to have a greatest length within this range. Taking the group as a whole, about one-half of them are under 13 mm. long and the other half measure 13 mm. or more. On the basis of their inner structure, the largest embryos are older members of the group and belong on the border line. Likewise, the 3 small ones belong among the youngest members of the group. One can speak less precisely of the size of the chorionic sac than of the size of the contained embryo. The available records for chorions are neither as complete nor as numerous as those for the embryos. If we omit the manifestly abnormal chorions, none of them have a greatest diameter of less than 30.0 mm., and one-third of them have greatest diameters of 40.0 to 48.0 mm. These ranges differ little from those in the preceding age group, but they are somewhat smaller than those found in horizon xviii.

As to the age of the members of horizon xvii, the estimate will again be based on matched macaque embryos of known ovulation age. Utilizing that procedure, it is found that macaque embryo no. C 479 of the Carnegie Collection,[3] 11.5 mm. long and 34 days old, corresponds closely to the embryos in the younger half of this age group, both in external form and in the status of its inner organogenesis. Macaque embryo no. C 488, 36 days, is described in the same paper. It has not yet been sectioned for microscopic study, but in outer form it matches some of the older members of this age group. In the light of these specimens the estimate of the ovulation age of embryos in horizon xvii falls between 34 and 36 days.

In summary it may be said that the chief characteristics which distinguish the external form of embryos in this horizon are the following: (1) The head is relatively larger than was seen in earlier age groups. (2) The main axis of the trunk has become straighter and there is a slight indication of a lumbar curvature. (3) The olfactory pit on each side has moved nearer the median line and is directed ventralward so that the opening of the nostril is not visible in profile views. Nasofrontal grooves are distinct. (4) The full complement of auricular hillocks is present on the mandibular and hyoid bars. The groove between these bars has become wider and has begun its transformation into the concha and external acoustic meatus. (5) The limb buds exhibit definite digital rays in the hand plate, and the foot has acquired a rounded digital plate set off from the tarsus and leg proper. (6) Surface elevations of individual somites are limited mostly to the lumbosacral region. In some younger members they can be seen at the lower thoracic levels. (7) In size, the embryos commonly range between 11.0 and 13.6 mm. greatest length. The chorionic sacs are 30.0 mm. or more in greatest diameter, with one-third of them ranging from 40.0 to 48.0 mm. (8) The ovulation age is estimated at $35 \pm 1$ days.

[3] C. H. Heuser and G. L. Streeter, Development of the macaque embryo, Carnegie Inst. Wash. Pub. 525, Contrib. to Embryol., vol. 29, pp. 15–55, see pl. 32, 1941.

## Coalescence of the Facial Growth Centers

A notable event in horizon xvii is the emergence of the outlines of the face. This takes place as a consequence of the enlargement and fusion of several growth centers which are generally spoken of as facial processes. As they merge, these produce structures recognizable as the definitive nose and upper jaw. The important steps in this transformation are illustrated in figure 1, xvii. Ever since the pioneer descriptions of His[4] they have been designated as facial processes. To call them that, however, has the disadvantage of oversimplification. In reality they are not prolongations having free ends which meet in the nasal region; nor is the ectoderm absorbed over their abutting surfaces. In transverse series of embryos the facial region is likely to be cut frontally, i.e. tangentially, which gives an exaggerated idea of their length. This is especially so in the series used by His (1901) and illustrated in figure 11 of his paper.

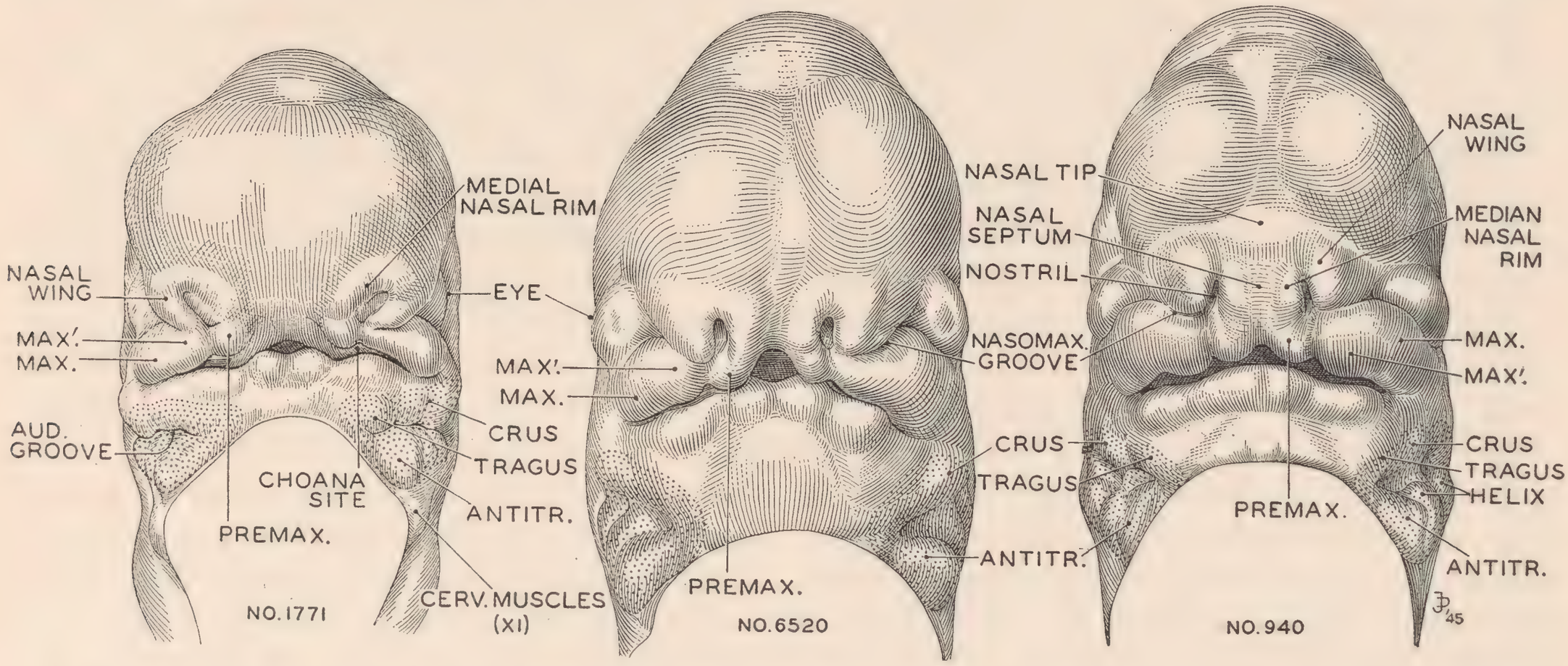



Fig. 1, xvii. This is the period of the emergence of the nose and upper jaw. Younger (1771), intermediate (6520), and older (940) representatives of horizon xvii are illustrated. Similar views of the preceding age group are shown in figure 2, xvi, and the following stage is shown in figure 1, xviii. All are magnified 12.5 times and can be directly compared. One can see the facial parts coming into existence as a consequence of the proliferation of the underlying mesenchymal growth centers. These undergo a coalescence of ridgelike masses, each mass differentiating into the various structures of its own region. Both the overlying epithelium and the precocious trigeminal and facial nerve strands appear to participate with the mesenchyme in the regulation of the form of the developing facial components. The field marked by the presence of the auricular hillocks is stippled, and the hillocks are labeled in accordance with the parts of the external ear derived from them.

Abbreviations: AUD. GROOVE, hyomandibular groove. As it becomes wider and deeper owing to the elevation of the surrounding structures, it is transformed into the concha and external auditory meatus. CERV. MUSCLES, cervical muscle center at tip of spinal accessory nerve. MAX., maxillary growth center. MAX'., supplementary maxillary growth center. MEDIAL NASAL RIM, medial nasal process. NAS. MAX. GROOVE, nasomaxillary groove. NASAL SEPTUM, ventral border of septum as it projects between the two nostrils. NASAL TIP, tip of nose as found in older members of group (no. 940). The nose at first is like a raised awning, and it will subsequently come down as its roof forms. NASAL WING, lateral nasal process. PREMAX., premaxillary or incisive center (global process of His). Drawings made by J. F. Didusch. Models made by O. O. Heard. × 12.5.

The alternate sections of this series are now in the Carnegie Collection, catalogued as no. 7317. It is more precise to speak of these structures as swellings or ridges which correspond to centers of growth in the underlying common mesenchyme. The furrows that lie between them on the surface are smoothed out as the proliferation and fusion of the growth centers fill in beneath. Under these circumstances no ectoderm requires absorption; it is simply flattened out

[4] W. His, Die Entwickelung der menschlichen und thierischer Physiognomien, Arch. f. Anat. u. Physiol., Anat. Abt., pp. 384–424, 1892.

*Idem,* Beobachtungen zur Geschichte der Nasen- und Gaumenbildung beim menschlichen Embryo, Abhandl. d. math.-phys. Classe d. Kgl. Sächs. Gesellsch. d. Wissensch., vol. 27, pp. 347–387, 1901.

in adaptation to the changed surface. This is in confirmation of the observations of Peter,[5] who found that the grooves separating the facial processes do not disappear as a result of the fusion of their edges. Instead they become more shallow and eventually smooth, as the increase in mass produces a new surface level. This point is to be kept in mind in analysis of the factors involved in the deformities that accompany harelip.

In the formation of the face certain aspects stand out as fundamental and require emphasis. In the first place, the component growth centers are bilateral, and the right and left groups are originally widely separated from each other. Secondly, these growth centers on each side include: (1) a ventromedial extension of the maxillary arch (maxillary process); (2) the ridge, or lip, forming the dorsolateral boundary of the olfactory pit, destined to become the nasal wing on that side (lateral nasal process); (3) the rounded medial rim of the olfactory pit (medial nasal process), which subsequently in cooperation with its mate and the intervening tissue unites in the formation of the septal elements of the nose, together forming a single mid-line structure, the composite nasal septum; (4) the growth center at the ventral end of the medial nasal rim, but discrete from it, destined to form the premaxilla (premaxillary center). The last is the globular process of His and is commonly included as part of the medial nasal process. It could be termed equally well the intermaxillary or incisive center. The term frontal process has been applied to the mid-line field lying between and dorsal to the olfactory pits. In the present age group the frontal field is marked by the absence of any condensation activity, and it is premature to speak of a "process." The latter will come considerably later as a part of the building of the elongated back of the nose. Thirdly and lastly, the above-mentioned growth centers blend with each other and, in some cases, meet across the median line. As they do so they exhibit in each instance the ability to form the various structures and strata required in their particular region, such as lip, alveolar and palatine processes, and eventually teeth.

It is in the present age group that the primitive face may be said to become established, but for a better understanding of the steps in the process one should compare what is seen in figure 1, xvii with the conditions present in the preceding horizon, figure 2, xvi, and subsequent one, figure 1, xviii, all of which are provided with uniform labeling and detailed descriptions which need not be repeated here.

## Primary Nasal Passages and the Respiratory By-Pass

The transformations of the right and left olfactory pits lead to the establishment of bilateral respiratory passages which by-pass the mouth, and their description properly follows that of the face. Already at horizon xvi the epithelium of the olfactory pit shows evidence of regional specialization. Because of this and the accompanying proliferation of the underlying mesenchyme, one can now recognize the outlines of the wings of the future nose and the median rim of each nostril.

From the edges of the nostril, and sharply demarcated from the abutting skin ectoderm, the olfactory epithelium extends backward into the preoptic region as a flattened pocket, constituting the nasal sac. Along the ventral fold of the latter, the epithelium exhibits a characteristic growth activity. It proliferates in the form of a platelike fin or keel which maintains an epithelial continuity between the nasal sac and the roof of the mouth. The location of this nasal fin is indicated on the surface by a groove, at first shallow, later becoming deeper and marking the boundary between the premaxillary and maxillary growth centers. This is the primitive palatine groove of Peter.[6]

Except for that maintained by the nasal fin, the continuity between the nasal sac and the roof of the mouth becomes interrupted by the active proliferation of the mesenchyme of the premaxillary and maxillary growth centers, which blends across from one center to the other, in front of the nasal fin. There is thereby established the primordium of the palate. The right and left primary palates are derived from the premaxillary centers and unite in the mid-line. That part of the palate derived from the maxillary centers appears a little later, taking the form of lateral elevations on the two sides.

[5] K. Peter, Atlas der Entwicklung der Nase und des Gaumens beim Menschen, pp. 6–8, Jena, 1913.

[6] *Ibid.*, figs. 6*a*, 11*a*, also text p. 9.

The developmental function of the nasal fin begins to express itself already in horizon xvi, at which time there is scarcely more than an olfactory pit. In horizon xvii the primary palate makes its appearance, and the nasal fin becomes transformed from an epithelial plate to an epithelium-lined passage in consequence

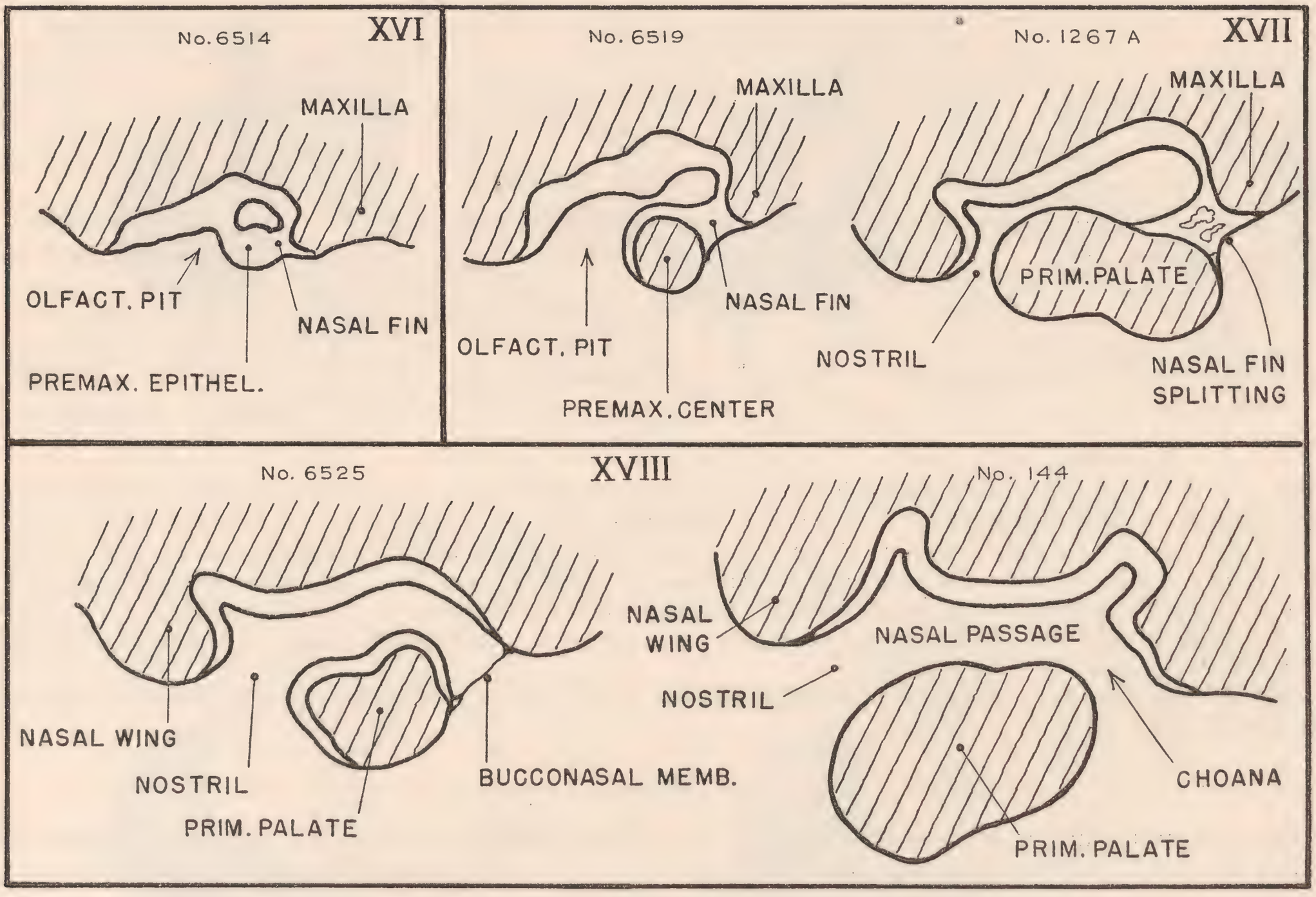



FIG. 2, xvii. Simplified outlines of selected sagittal sections showing the steps in the formation of the respiratory by-pass in horizons xvi, xvii, and xviii. Judging from its prominence and precocity, the epithelium of the olfactory pit appears to be an important determining factor. This same epithelium is a mosaic of at least four specialized areas. One forms the zone of olfactory cells; another becomes the vomeronasal organ (Jacobson's); another participates in the formation of the turbinate system; and another is the platelike nasal fin, which by a characteristic splitting phenomenon produces the choana. The underlying mesenchyme is an important partner in these changes. It is from the maxillary and premaxillary centers that the primary palate spreads forward and across, thereby separating the nasal passage from the mouth. The olfactory ectoderm is much thicker than the contiguous skin ectoderm, but allowance is to be made for exaggeration in some places due to the tangential direction of the section, notably in the first two stages. In each case the outlines represent camera lucida tracings of single sections, with the exception of the last (no. 144), which is a composite of two sections. ×40.

of the coalescence of its cleavage spaces and through adaptation of its rapidly proliferating cells. As long as the end of this passage is obstructed by incomplete cleavage of the epithelium, it constitutes a cul-de-sac, the "hinteren Blindsack" of Peter. In horizon xviii, among the younger members of the group, an epithelial membrane-like remnant (the bucconasal membrane of Hochstetter) still stretches across the opening. In older members, the last strand has been retracted and a free respiratory passage exists from the nostril through the choana to the pharynx. The steps in the laying down of the primary nasal passage can be seen in simplified form in parasagittal sections, as is illustrated in figure 2, xvii. Sections taken from four embryos belonging in horizon xvii are shown in greater detail in figure 3, xvii. These illustrate the range of development present in this age group. They illustrate the nasal fin during its maximum prominence, as seen in both transverse and sagittal sections.

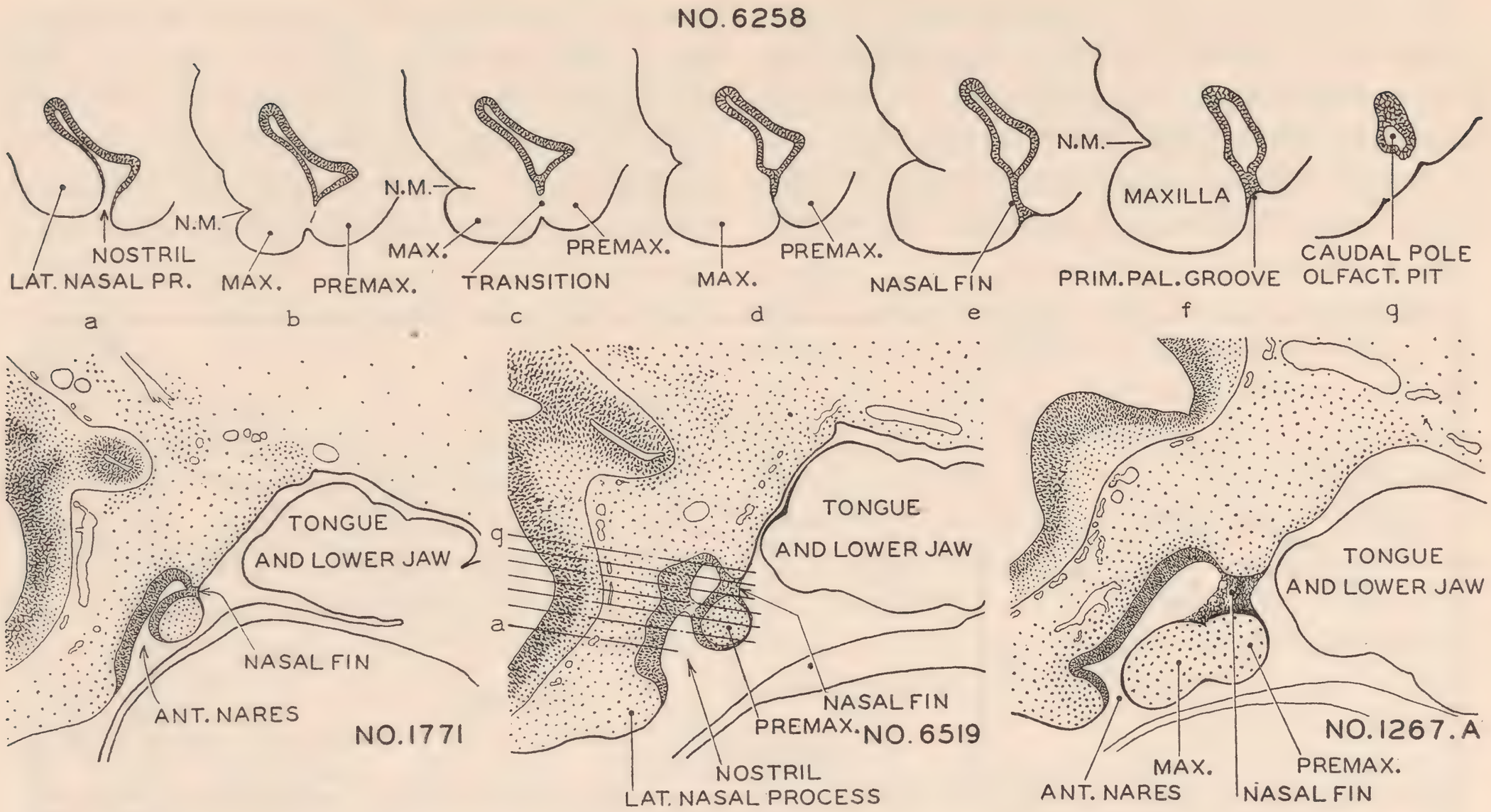



FIG. 3, xvii. Upper row, camera lucida drawings of transverse sections (in most instances, every fifth section) through right olfactory pit of embryo no. 6258. This embryo corresponds closely to no. 6519, a sagittal section of which is shown in the lower row. To facilitate comparison of the two, the approximate section levels of the tracings of no. 6258 are superimposed upon the latter. The sagittal drawings in the lower row show younger (no. 1771), intermediate (no. 6519), and older (no. 1267A) representatives of this age group, a period of rapid transformation in the nasal region. It is to be noted that the primary characters of this respiratory by-pass are acquired in miniature and before the attainment of its place relation to the tongue.

LAT. NASAL PR., nasal wing; MAX., maxillary growth center; N. M., nasomaxillary groove; PREMAX., premaxillary growth center. ×25.

## HEART

This age group brings with it three notable advances in the structure of the heart, namely: (1) the advancing cleavage of the primary heart tube, separating the pulmonary and aortic channels; (2) the initiation of the aortic and pulmonary semilunar valves; and (3) complete separation of the right and left atrioventricular canals. As can be seen from the sections shown in figure 4, xvii, the primary heart tube and its surrounding gelatinous reticulum still form a considerable part of the field and stand out in contrast with the trabeculated muscular sacs that are forming the right and left ventricles. As has been pointed out, the right and left ventricular pouches, as they increase in size and pulsating force, tend to give rise to two diverse currents corresponding to their separate directional axes. Apparently this finds its expression in the expansion of the endothelial lumen into two main channels (aortic and pulmonary), as seen in embryos nos. 3385 and 6510 in figure 3, xv. The endothelium between them is consequently subject to less distention, resulting in its final absorption. Thereupon the supporting gelatinous reticulum fills in along that spiral path of less stress. Under such circumstances the adaptation of endothelium to the existence of two blood currents must rank high as a factor in the cleavage of the aortic and pulmonary trunks, whereas the ingrowth of the right and left bulbar ridges of the supporting gelatinous reticulum may be merely subsidiary, a filling-in behind the reshaping of the endothelium. One finds at this time, directly underlying the endothelium, an increase in the supporting reticular cells. Such a condensation is found particularly in the plane in the reticulum where the aortic and pulmonary channels are undergoing cleavage. An area of that kind is illustrated in section 6-2-2 in figure 4, xvii, and it signifies that one is close to an endothelial surface.

In an organ with complicated cavities, such as char-

acterize the heart at this time, their form and communications can be studied with advantage in three-dimensional reconstructions, like the one shown in figure 5, xvii. In the embryo which was selected for this purpose the atria were contracted, and this made it favorable for viewing the two atrioventricular left dorsal aorta. It also shows the progress of cleavage of the endothelial aortic and pulmonary trunks, to which reference was made in the preceding paragraph. We now have something more than the formation of two separate trunks, namely a specialization of the endothelium and its supporting gelatinous

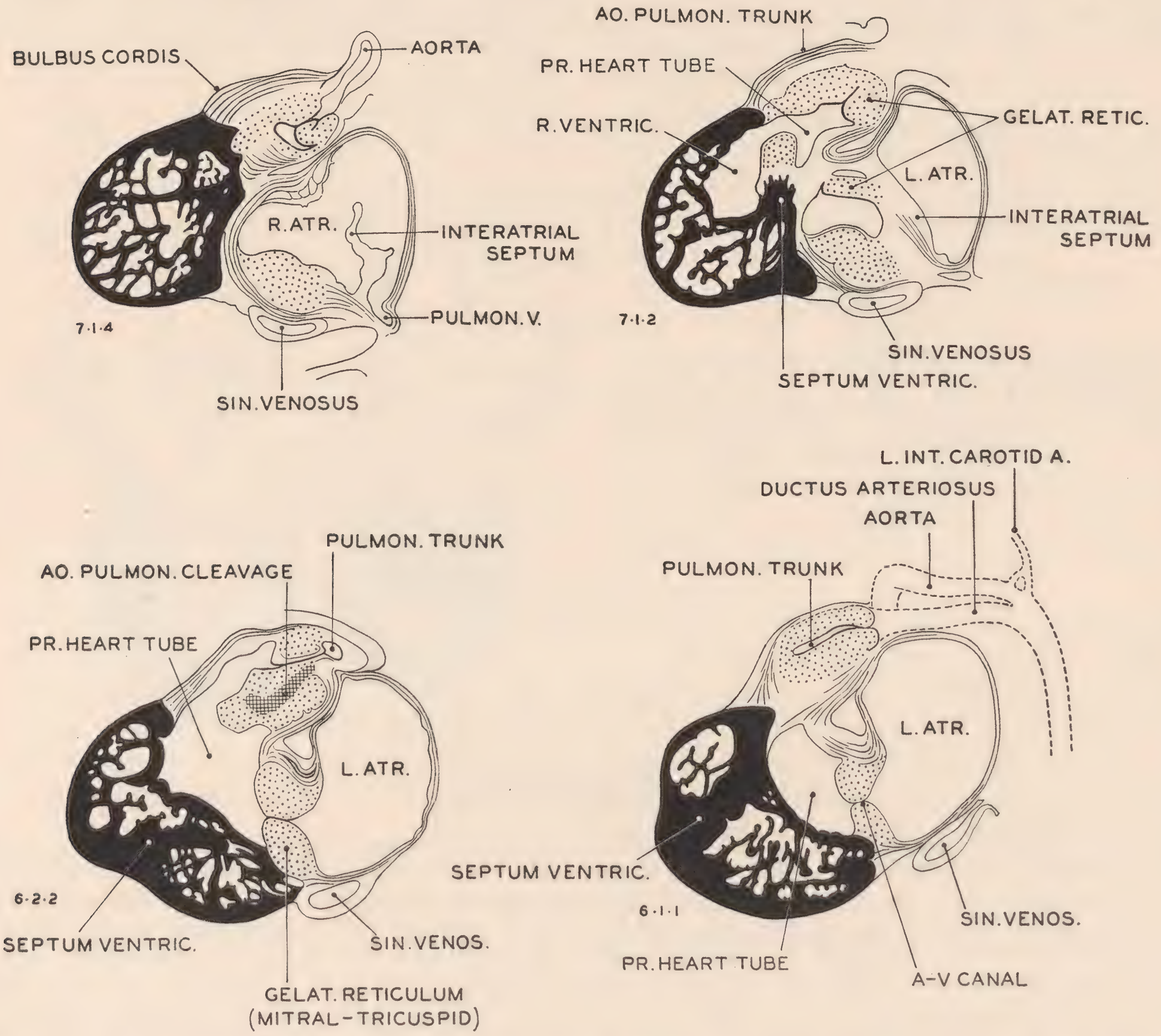



FIG. 4, xvii. Four selected sagittal sections through the heart of embryo no. 1771, a younger member of age group xvii. The aortic and pulmonary trunks are undergoing cleavage, a process which starts peripherally and extends backward into the heart. At this time the trunks have been separated to a point proximal to the semilunar valves, which are now present in crude form. ×30.

canals.[7] The model is shown in two views, or at two levels, one (A) being in front of and to be superimposed upon the other (B). The front model (A) shows the pulmonary and aortic trunks leaving the heart, and the part they take in the formation of the reticulum into the aortic and pulmonary semilunar valves. At these places the endothelium preserves the continuity of the lumen, whereas the condensing reticulum fills in behind it, forming three characteristic rounded occluding masses. The narrow lumen through the valve appears on section as a three-limbed cleft. This, therefore, is technically a weak spot, and when one attempts to make a cast of the lumen of

[7] The reader is referred to T. C. Kramer's paper on the partitioning of the human heart, particularly his figures 6 and 7, Amer. Jour. Anat., vol. 71, pp. 359–361, 1942.

these trunks it is likely to result in a break at the semilunar sites. Proximal to the valves the primary heart tube is still relatively voluminous, and the larger parts correspond more or less to channels suited to the two main blood currents. In the formation of the semilunar valves, if mechanical factors enter in, they certainly must be much more complicated than those suggested as underlying the separation of two main

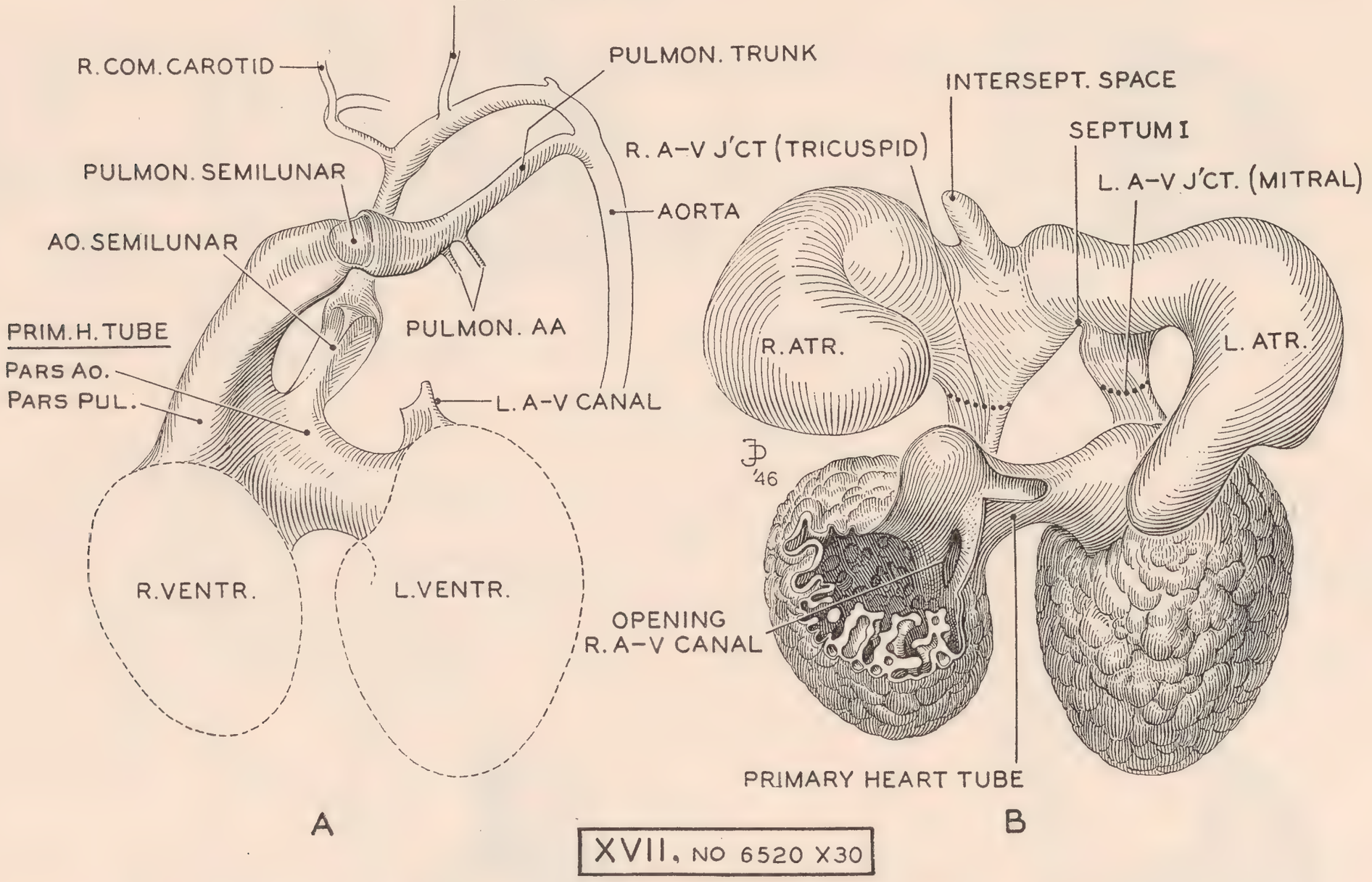



FIG. 5, xvii. Reconstruction model of the endothelium of the heart and its arterial trunks in embryo no. 6520, a member of the median third of horizon xvii. It is shown in two parts, A and B. In A, only the derivatives of the primary heart tube are shown. In B, enough of the primary heart tube is removed to expose the posterior or venous part of the heart. The latter, being in a contracted state, reveals unobstructed the right and left atrioventricular canals, now clearly separated, and each leading forward into its respective ventricle. It will be noted that the primary heart tube is connected with the venous part of the heart only at the two atrioventricular junctions. When the latter are severed, the ventral arterial heart can be freely separated from the dorsal venous heart. In part A the aorto-pulmonary cleavage has not yet crossed the crest of the septum, and both arterial trunks lead off from both ventricles. From the distention of the endothelium of the primary heart tube, however, one can see that the left ventricle favors the aorta, and the right ventricle the pulmonary trunk. Drawings made by J. F. Didusch. Model reconstructed by O. O. Heard. ×30.

blood channels. But even in these valves one would hesitate to rule out the possibility of there being some mechanical influence at these sites, associated, for instance, with the back-thrust of the systemic blood columns which would, already at this time, follow each heartbeat.

The posterior model (B) shown in figure 5, xvii is visualized as dissected free from the more anterior-lying part (A). The cut edge of the primary heart tube (B) is arranged so as to leave uncovered the entrance of the right atrioventricular canal. From examination of both views (A and B) one finds that the separation of the aortic and pulmonary channels is complete to the region proximal to the semilunar valves. From there backward, the primary heart tube still consists of a single voluminous cavity, common to right and left sides. Into it, by two slitlike openings, the incoming blood enters separately from the right and left atria. These openings, or canals, mark the sites of the future mitral and tricuspid valves, whose primordia are still in the form of a condensing gelatinous reticulum. With this kind of heart, having a simple form of semilunar valves but still without mitral and tricuspid valves, embryos of horizon xvii are able to maintain a blood circulation adequate for their needs.

## Lung

The separation of the lung from its mother system, the gut tract, is in this age group essentially complete up through the laryngeal region. It should, therefore, be considered separately, and this can be done briefly. The form of the epithelial bronchial tree is illustrated in figures 6 and 11, xvii. It will be seen that the primary branches from the stem bronchi now have secondary branches, and one can more definitely recognize the groupings that will make up the three lobes on the right side and two on the left. In addition to the growth and branching of the epithelial tube, the surrounding visceral mesenchyme, which, in part at least, was derived from the coelomic epithelium, is advancing in its differentiation, and one can now recognize condensations that are to become tracheal cartilages. There has been a progressive increase in size of the vagus nerves as compared with those at a comparable level in the preceding age group. These points can be seen in the section of an embryo of this age group shown earlier with specimens of other horizons in figure 27, plate 3, xv. It will be noted in the photograph that the esophagus lying behind the trachea is acquiring a reticular coat enclosing the epithelium, which coat is practically absent from the trachea. It is also to be noted that the right and left pulmonary arteries have become more prominent.

## Gut Tract

In the accompanying figure 6, xvii, there is shown in outline the epithelial gut tract that is characteristic of this age group. As heretofore, it is the prime determining tissue of the alimentary system and maps out the component elements. Over and above its relative increase in length, various regional morphological details are making their appearance. In addition to a caecum, one can now distinguish the vermiform appendix. The primary intestinal loop projects farther into the umbilical cord. Rotation of this loop and its supporting mesentery has not yet occurred. The elongating colon, however, is providing the thrust that will aid in bringing the caecum to the right side of the body.

The duodenal region is an especially active center of epithelial proliferation. This proliferative activity results in two fusiform enlargements, one above the entrance of the bile and pancreatic ducts and one below. In the region of these enlargements the epithelium in its accentuated growth acquires a follicular arrangement in which the epithelium is oriented around small discrete cavities. In some cases these constitute isolated follicles and in others they consist of diverticulae, communicating with the partially obliterated original lumen of the gut (fig. 7, xvii). Traces of this exuberant type of epithelial proliferation with its characteristic follicular tendency are found also in other parts of the gut tract, notably in the esophagus.[8]

The relations of the ventral and dorsal pancreas and of the biliary ducts to the gut tract require a detailed sketch, such as is shown in figure 8, xvii. In it one can see that the ventral pancreas, which had sprouted off from the common bile duct, has now fused with the dorsal pancreas. Each pancreas, however, still retains its original duct, that of the ventral pancreas emptying into the common bile duct and that of the dorsal pancreas, higher up, directly into the duodenum. Eventually, following anastomosis of the duct systems of the two parts, the more favored tract for the pancreatic secretions from the whole gland leads through the ventral pancreatic duct alongside the bile duct, and, as a rule, the dorsal pancreatic duct disappears in the process.

## Liver

Back in an earlier horizon, we have seen the liver take form as the joint product of angioblastic tissue arising from the coelomic surface cells (pl. 2, xi) and of epithelial columns coming from the opposite direction, sprouting off from the hepatic evagination of the gut epithelium (fig. 3, xii). The two tissues in this combination undergo continued rapid proliferation, and there is soon produced the typical large trabeculated epitheliovascular organ which we designate as liver, and which shows signs of functional activity of importance to the embryo from the begin-

[8] This peculiarity of the early duodenal epithelium was described in detail in the excellent study of F. P. Johnson, Amer. Jour. Anat., vol. 10, pp. 521–561, 1910.

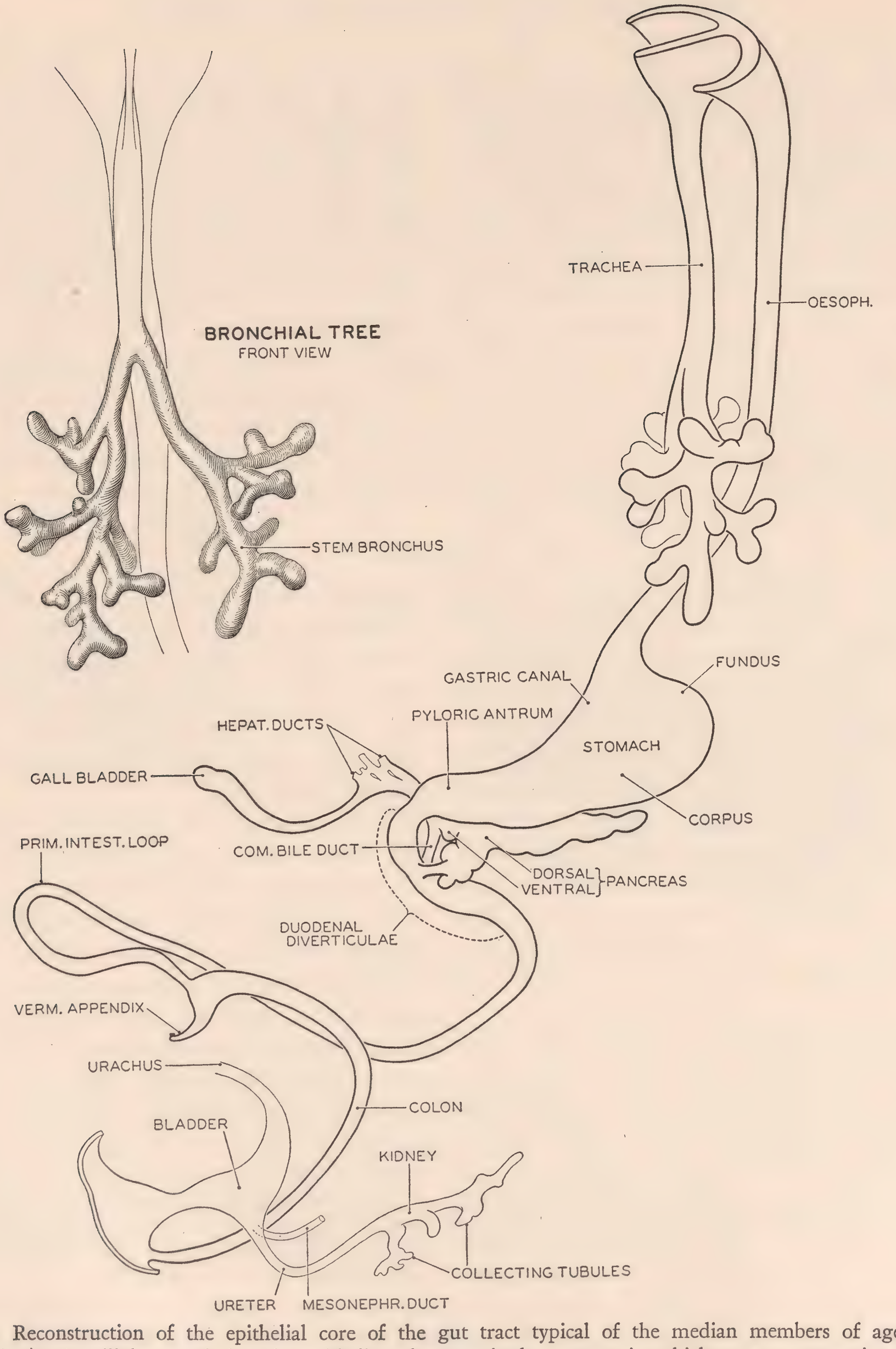



Fig. 6, xvii. Reconstruction of the epithelial core of the gut tract typical of the median members of age group xvii (embryo no. 6520). It will be noted that the epithelium has acquired a pattern in which one can recognize many details approximating the regional forms of the mature tract, and looking back at the previous horizon (fig. 5, xvi) one can see that the elements of this epithelial pattern were already present at that time. Again, antedating that was a level with simpler expression of the same parts, and so back to the primordial system. Thus starting from its primary form this precocious epithelium of the gut progresses step by step, always in the direction of its inherent plan and at no place deviating from it. Considering the relative backwardness of the other coats of the gut tract during this period, one must attribute a high degree of intrinsic potency to the different regions of the gut epithelium.

It will be noted that in the present age group the vermiform appendix becomes marked off from the caecum. In the kidney the pelvis has become branched. The primitive intestinal loop herniates forward into the umbilical cord without rotation or coils. Drawing made by J. F. Didusch. Reconstruction made by O. O. Heard. ×33.4.

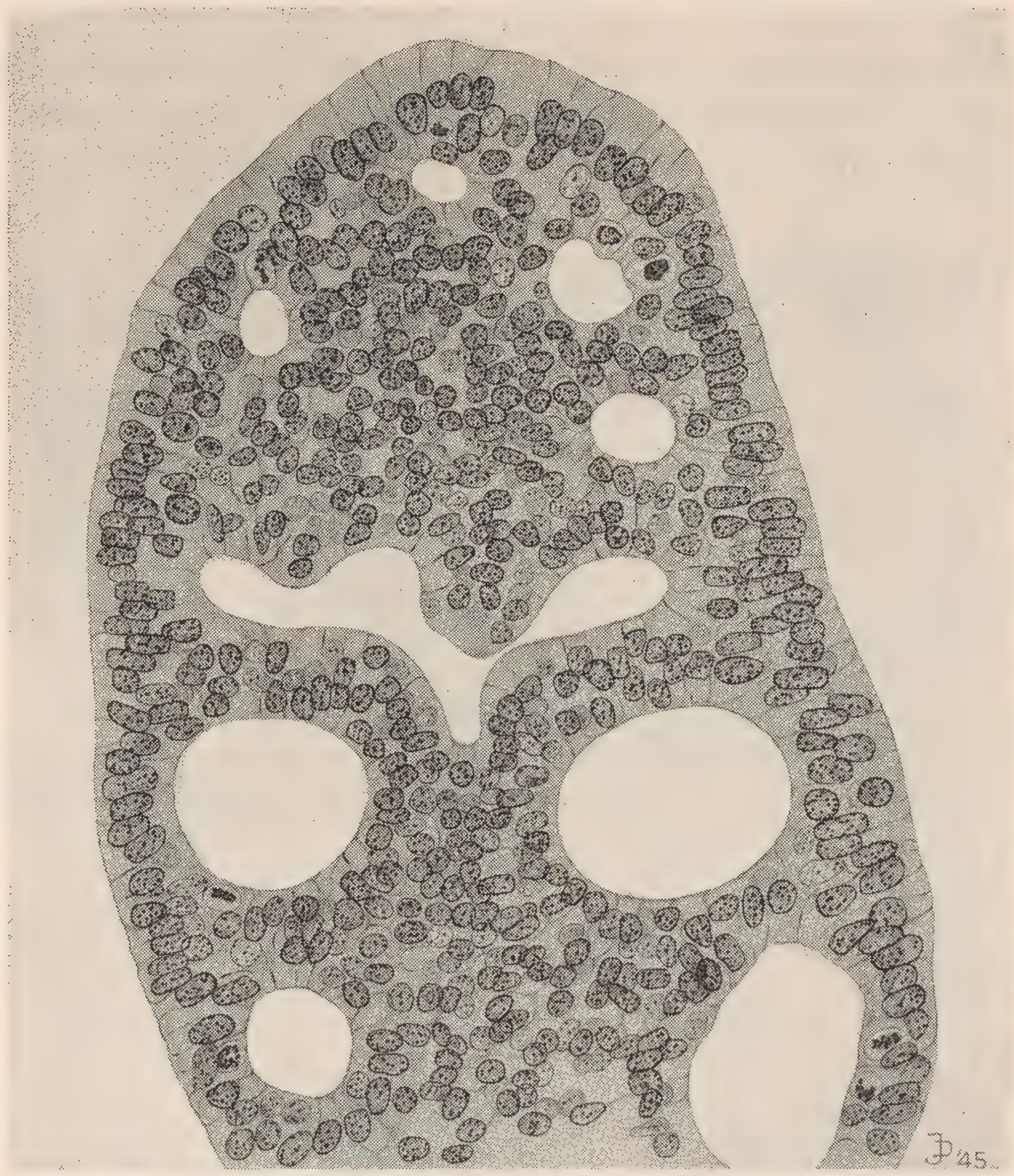


FIG. 7, xvii. Section through duodenal epithelium of same specimen whose gut tract was shown in figure 6, xvii. In its proliferative activity it forms diverticula opening from the central lumen and also isolated follicles. Because of the exuberant growth of its epithelium there are produced the two spindle-shaped enlargements of the duodenum, above and below the combined pancreatic and biliary ducts. Drawing made by J. F. Didusch. Section 48-3-3. ×400.

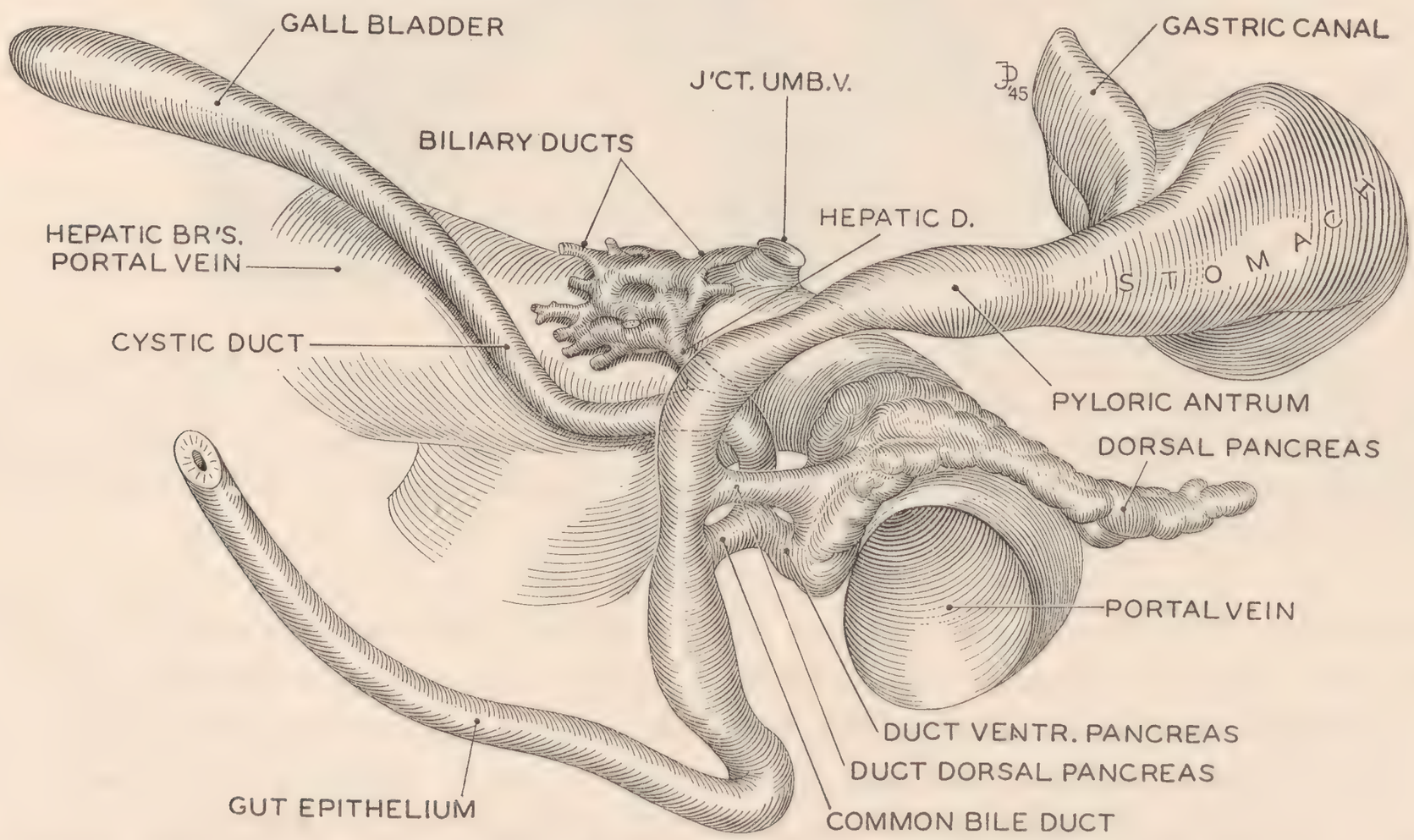



FIG. 8, xvii. Reconstruction of the epithelial core of the two pancreatic ducts; showing also the biliary ducts and their relation to the gut tract epithelium, typical of age group xvii (embryo no. 1267A). It can be seen that the dorsal pancreas, though fused with the ventral pancreas, still retains its original duct. Drawing made by J. F. Didusch. Reconstruction made by O. O. Heard. ×50.

ning of blood circulation and thereafter. If its first functional assignment were to be its sole function, there appears to be no reason why it should not be-

hepatic duct is illustrated in figure 9, xvii.[9] It does not penetrate far into the liver substance as yet, and is found only at the hilum in the connective tissue

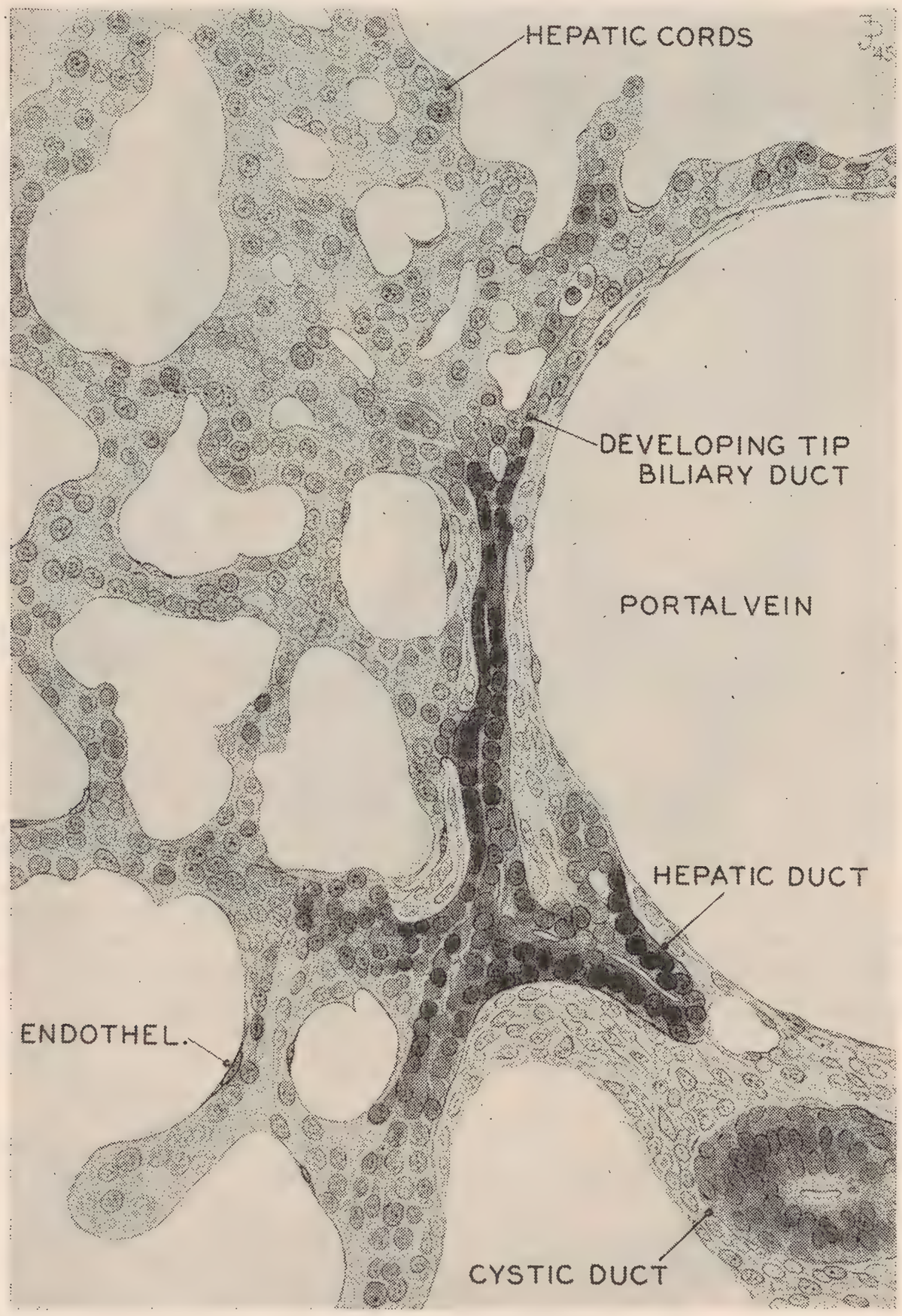



FIG. 9, xvii. Histological appearance of the abrupt transition from the biliary ducts to the hepatic epithelium. It supports the assumption that at the tips of the ducts some of the hepatic epithelium becomes redifferentiated into the compact, deeply staining duct-forming cells. This process of redifferentiation progresses outward through the embryonic connective-tissue coat of the portal vein and its branches. The growth of new connective tissue and the duct formation keep in close step. Drawing made by J. F. Didusch. Embryo no. 1267A, section 5-1-1. ×250.

come detached from the gut wall and carry on by itself. It does not, however, become detached. There remains an epithelial stem which preserves the continuity between the liver epithelium and that of the gut. This stem is partially plexiform, and by the time the present horizon xvii is reached it is readily recognized as the hepatic duct.

The nature of the abrupt terminal endings of the

of the wall of the entering portal vein. At its elongating tips, it is directly continuous with the liver epithelium, and its further peripheral growth is accomplished at the expense of the latter, which cell by cell at these tips is transformed into smaller dark-

[9] This figure corresponds closely to the photograph published by W. Bloom (Amer. Jour. Anat., vol. 36, p. 459, 1926), to whose important observations on the embryogenesis of bile capillaries the reader is referred.

staining duct cells. This transdifferentiation keeps step with the formation of the connective-tissue coat of the portal vein, along whose branches the biliary duct system spreads. Near the hepatic duct, but essentially separate in origin, is the gall bladder and its duct. These are in the nature of specializations and do not have the basic significance of the hepatic duct itself. The hepatic duct and the plexiform terminations of its biliary tributaries, in the reticulum along the wall of the portal vein, have been modeled in three dimensions and are shown in figure 8, xvii.

## Central Nervous System

During the two days that have been added to the progress of the embryo since the preceding age group, the brain has acquired a more definite appearance of subdivisions. The basal plate of the hypoglossal region is now more or less continuous with the tegmentum of the oculomotor region. The rhombic grooves are pressed to the side and absorbed in the surrounding surface, so that they are no longer visible in a median sagittal view, such as is shown in figure 10, xvii. In general, it is the basal parts of the neural tube that are taking the lead. The subthalamic region, lying below (i.e. rostral to) the marginal ridge, is well in advance of the thalamus itself. The hypothalamic region is also relatively precocious. The corpus striatum is now sharply demarcated, and the olfactory evagination has made its appearance. The evagination of the infundibular floor in the form of a wrinkled sac has now advanced far enough to be recognized as the neural hypophysis (posterior lobe). On the lateral surface of the hypothalamic region

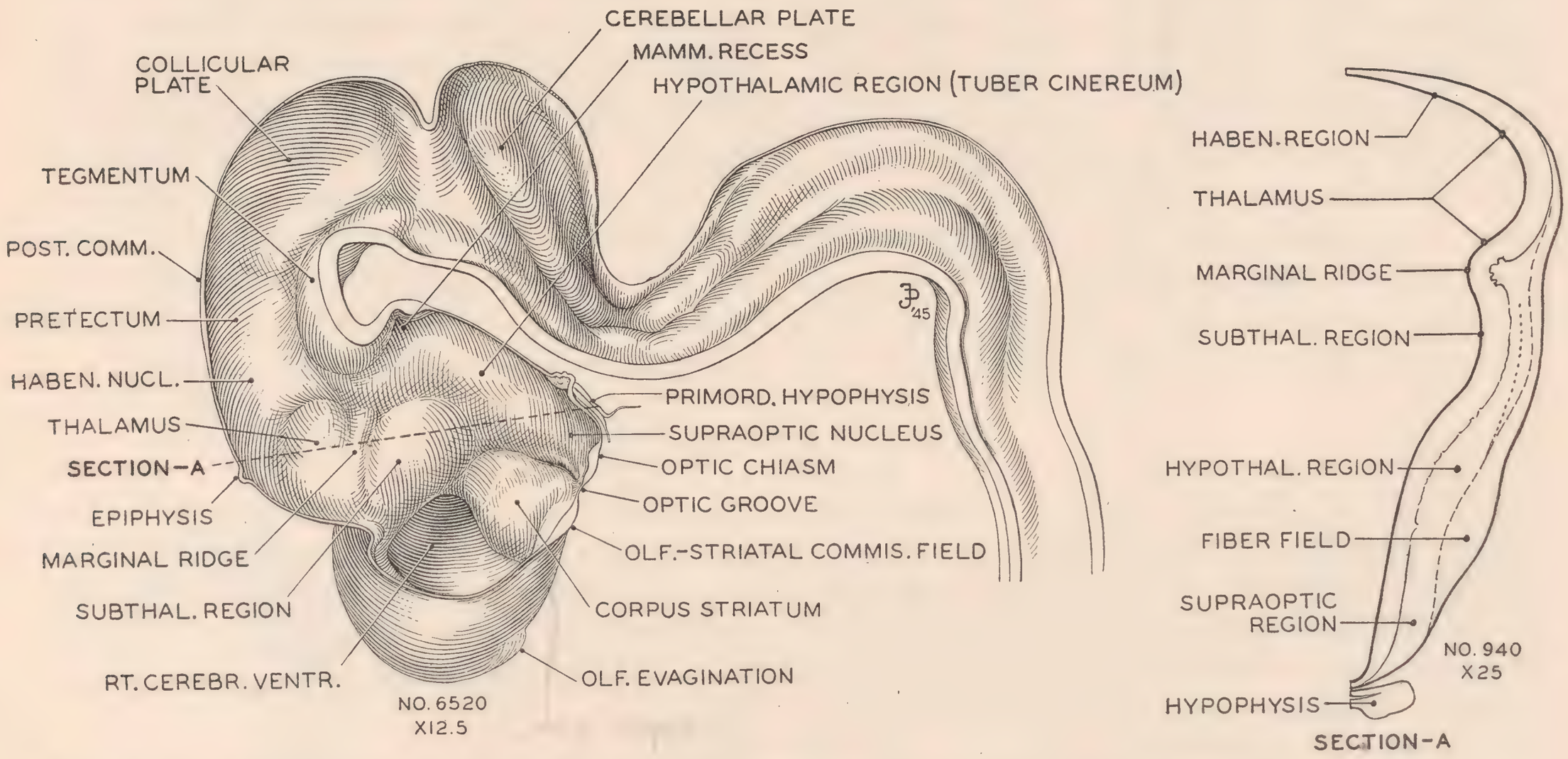



Fig. 10, xvii. Reconstruction of right half of brain of an embryo (no. 6520) belonging in the middle third of age group xvii. The marginal ridge, projecting into the lumen and separating the thalamus from the subthalamic region lying rostral to it, is now plainly seen. The contours of the wall are better seen in section A. This drawing was made from embryo no. 940, of similar age, and it corresponds to a transverse section along the dotted line shown in the sketch of the reconstruction, passing caudal to the epiphysis, with its lower end transecting the hypophysis. It will be noted that above the marginal ridge the wall is backward in development, and ventral to it the wall is precocious. The subthalamic region has a nuclear area comparable to a mantle zone. In the hypothalamic region there is a large fiber field which appears to unite later with the peduncular system. The neural hypophysis forms a deep ventral pocket with characteristic foldings of its caudal wall. The anterior lobe is flattened anteroposteriorly, but spreads widely, enclosing the neural lobe on each side with its two wings. It is therefore larger than is shown in a sagittal section. Finally, it is to be noted that the olfactory evagination is just making its appearance. Drawings made by J. F. Didusch. Reconstruction made by O. O. Heard. Reconstruction ×12.5. Section (no. 940, 13-1-1) ×25.

there is now a prominent fiber field which is interpreted as being one of the series of components that eventually join to form the great peduncular tract. See embryo no. 940 in figure 10, xvii. The roof parts, collicular plate, pretectum, habenular region, thalamus, and neopallium, though rapidly expanding as relatively thin walls, show scant advance in differentiation.

*Eye and Ear*

Even under low power one can now clearly see the granules of retinal pigment in all parts of the outer layer of the retina, as is illustrated in figures 31 and 32 on plate 3, xvii. Because of increase in length of lens fibers in the lens body, the cavity of the vesicle is becoming crescentic in section. In the cavity, there are still seen the remnants of disintegrating cells. A noteworthy event in this age group is the migration "inward" of some of the retinal cells and their alignment in the form of the inner nuclear layer. An early phase of this migration, in a well preserved specimen, is shown in figure 31, plate 3, xvii. The phenomenon begins in a rather definite region. As one faces the eye ground, the center of the migration is lateral to and somewhat above the site of the optic papilla.[10] That is, it is not far from where the macula lutea will eventually be found. If we may liken the primary nuclear layer of the retina to the ependymal zone of the early spinal cord, the migration "inward" of the inner nuclear layer is comparable to the formation of a mantle zone.

The type of membranous labyrinth characterizing this age group is shown in figure 11, xvii (embryos nos. 485 and 6631). The endolymphatic appendage is a relatively large thin-walled fusiform sac. The cochlear duct is actively elongating at the tip of the labyrinth. But the most significant change is the thinning out and approximation of the walls of the vestibular part of the labyrinth, making imminent the absorption of the contacting surfaces with resultant sealing off of the three marginal thickened rims which are destined to serve as the semicircular ducts. In this age group, only those specimens are admitted in which the absorption has not yet occurred, and consequently in none of them is a semicircular duct present.

## Summary of Horizon XVII

In review of the inner structure of embryos in horizon xvii, there is shown in figure 11, xvii an outline sketch of organ forms which one can easily verify by microscopic examination of ordinary serial sections of a given embryo. These characters were not present in any of the previous horizons and, within the range of variation illustrated, they are present in all the specimens listed in the table of embryos of this horizon.

In addition to the items in this syndrome, it may be added that in the heart one finds cleavage of the pulmonary from the aortic trunk to a level proximal to the aortic and pulmonary semilunar valves, which are now present. Also there is now complete separation of the right and left atrioventricular canals.

In the gut tract, it is to be noted that the vermiform appendix is present and that the dorsal and ventral pancreas have fused, but still retain their individual ducts. In the liver one finds the beginning invasion of bile ducts along the surface of the portal vein, arising by redifferentiation of the contacting hepatic epithelium.

In the central nervous system the development is more precocious along the basal parts of the neural tube, notably in such structures as the tegmentum, subthalamic and hypothalamic regions, and corpus striatum. The olfactory evagination is now definite, and the neural lobe of the hypophysis is laid down.

[10] A series of reconstructions showing the progressive differentiation of the retina and eye ground in human embryos is illustrated in figure 109 in the well known book by Dr. Ida C. Mann, Development of the human eye, Cambridge, England, 1928.

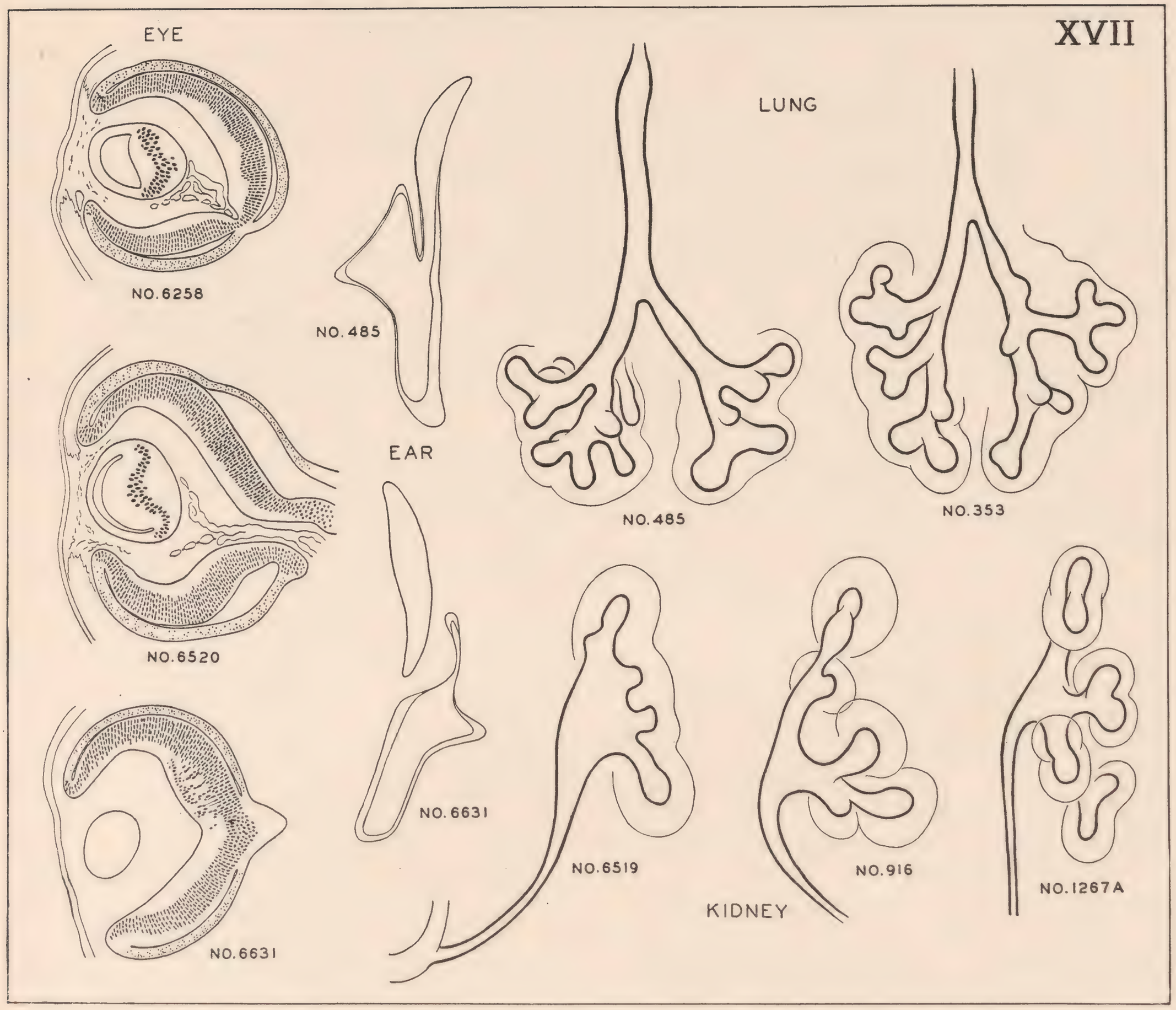



FIG. 11, xvii. Syndrome of structural characters required of members of age group xvii. Migration of retinal nuclei as the first representatives of the inner nuclear layer of the retina. At this time the migration is limited to a restricted field, approximately at the site of the macula lutea. The cavity of the lens vesicle, as seen in section, shows various stages in becoming a crescentic cleft. In the inner ear the walls of the labyrinth, save at its thick rims that are to be the ducts, are beginning to sink inward, preparatory to absorption of their thin parts. In the lungs the branches of the stem bronchi are acquiring branches of the second order. The pelvis of the kidney begins to exhibit well marked calyces. Eye and kidney $\times 66.6$; ear and lung $\times 33.3$.

## Normal Embryos in Carnegie Collection Belonging in Horizon XVII

The explanatory notes first supplied under age group xiii and followed in subsequent horizons apply also here. In the case of each embryo marked with an asterisk (*), the length as recorded in the laboratory has been adjusted to an estimated standard, so as to be more nearly comparable with the lengths of other embryos in the collection. Other embryos are added when acquired.

### List of Embryos

| Serial no. | Size (mm.) | Grade | Fixation | Cutting medium | Plane | Thinness (microns) | Stain | Date, donor, and remarks |
|---|---|---|---|---|---|---|---|---|
| 353.. | E., 11.0<br>Ov., 40 × 35 × 20 | Good | Formol | P | Frontal | 10 | H.-E. | 1906, M. Brödel, Baltimore<br>Near border line group xviii |
| 485.. | E., 13.0<br>Ov., 33 × 25 | Exc. | Formol | P | Frontal | 40 | Al. ćoch. | 1911, G. L. Hunner, Baltimore<br>India ink injection |
| 544.. | E., 11.5<br>Ov., 30 | Good | Zenker formol | P | Sag. | 40 | Al. coch. | 1911, G. L. Hunner, Baltimore<br>Operative; India ink injection |
| 559.. | E., 8.6<br>Ov., 20 × 15 × 12 | Good | Formol | P | Trans. | 20 | H.<br>Cong. r. | 1911, B. J. Merrill, Stillwater, Minn.<br>Cyclopean abnormality |
| 562.. | E., 13.0<br>Ov., 28 × 17 × 17 | Poor | Formol | P | Sag. | 100 | Al. coch. | 1912, W. A. Duvall, Baltimore<br>Old in group |
| 623.. | E., 10.1<br>Ov., ..... | Good | Alc. | P | Trans. | 20 | H.<br>Cong. r. | 1912, R. P. Batchelor, Milwaukee, Wis.<br>Operative; median place in group |
| 695.. | E., 13.5<br>Ov., 40 × 40 × 17 | Poor | Formol | P | Trans. | 10 | H.<br>Cong. r. | 1913, E. H. King, Portland, Me.<br>Maceration |
| 916 | E., 11.0<br>Ov., 30 × 30 × 16 | Good | Bouin | C | Trans. | 40 | H.-E.<br>or. G. | 1915, G. G. Rusk, Baltimore<br>In oldest third of group |
| 940.. | E., 14.0<br>Ov., 28 × 23 × 21 | Good | Formol | C | Trans. | 40 | H.-E.<br>or. G. | 1914, J. Girdwood, Baltimore<br>Near older border line |
| 1232.. | E., 14.5<br>Ov., 35 × 35 × 30 | Poor | Formol | P | Frontal | 40 | Al. coch. | 1915, H. Sewell, Denver, Colo.<br>Close to no. 1267A |
| 1267A | E., 14.5<br>Ov., 35 × 30 × 26 | Good | Formol | C | Sag. | 20<br>40 | H.-E.<br>or. G. | 1915, J. M. Melton, Crozet, Va.<br>Excellent for central nervous system |
| 1771.. | E., 12.5<br>Ov., ..... | Good | Formol | P | Sag. | 20 | H.-E. | 1917, H. M. N. Wynne, Baltimore<br>Tubal, showing implantation |
| 5642.. | E., 11.5<br>Ov., 33 × 30 × 17 | Good | Formol | P | Trans. | 15 | Al. coch. | 1928, W. Coleman, Baltimore<br>Mechanical injury right arm |
| 5893.. | E., 13.2<br>Ov., ..... | Good | Formol | C-P | Trans. | 20 | Al. coch. | 1929, A. Plaut, New York<br>Oldest in group |
| 6258.. | E., 14.0<br>Ov., 48 × 35 × 25 | Good | Formol | C-P | Trans. | 10 | H.-E. | 1930, W. M. German, Grand Rapids, Mich.<br>Median in group |
| 6519.. | E., 10.8*<br>Ov., ..... | Exc. | Corros. acetic | C-P | Sag. | 8 | Al. coch. | On border line of youngest and middle third |
| 6520.. | E., 14.2*<br>Ov., ..... | Exc. | Corros. acetic | C-P | Trans. | 10 | Al. coch. | Median place in group; suitable for photography |
| 6521.. | E., 13.2*<br>Ov., ..... | Exc. | Corros. acetic | C-P | Trans. | 8 to 18 | Al. coch. | Microtome out of order; sections of varying thicknesses |
| 6631.. | E., 13.0<br>Ov., ..... | Good | Formol | C-P | Frontal | 10 | H.-E. | 1932, A. H. Koff, Baltimore<br>Tubal; near older border line |

(*Continued on following page*)

LIST OF EMBRYOS—*Continued*

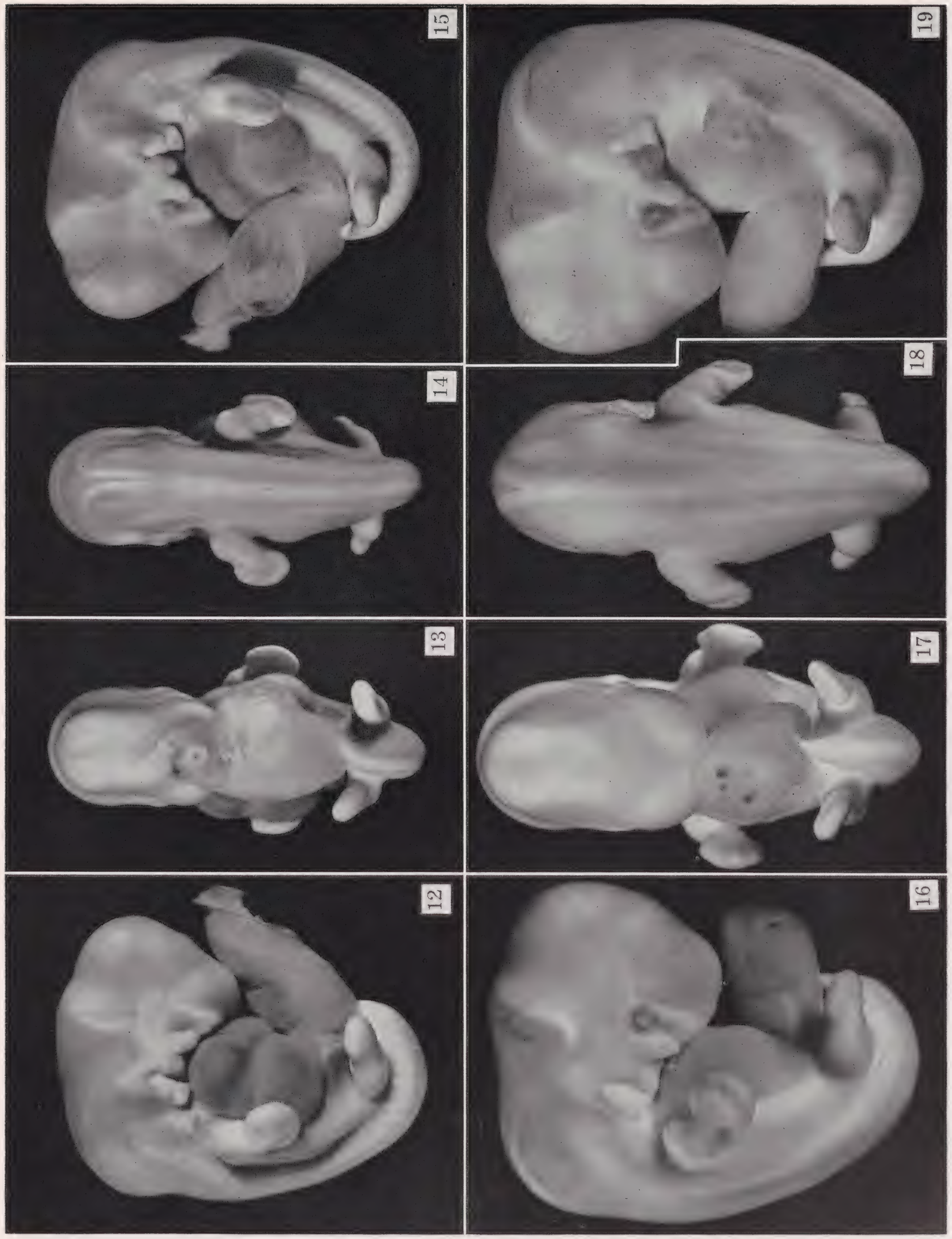
| Serial no. | Size (mm.) | Grade | Fixation | Cutting medium | Plane | Thinness (microns) | Stain | Date, donor, and remarks |
|---|---|---|---|---|---|---|---|---|
| 6742.. | E., 11.0<br>Ov., 50 × 40 × 15 | Good | Formol | C-P | Trans. | 12 | H. phloxine | 1933, J. L. McKelvey, Baltimore<br>Favorable for primary germ cells |
| 6758.. | E., 12.8<br>Ov., ..... | Good | Formol | C-P | Trans. | 10 | H. phloxine | 1933, J. Casagrande, Brooklyn, N. Y.<br>Among youngest third |
| 7317.. | E., 10.0*<br>Ov., ..... | Good | ..... | P | Frontal | 10 | H.-E. | 1936, Robert Meyer, Berlin<br>His embryo "Ru," every third section |
| 7436.. | E., 13.0<br>Ov., ..... | Good | Formol | C-P | Frontal | 30 | Al. coch. | 1937, M. P. DeVito, Washington, D. C.<br>Among oldest third |
| 8101.. | E., 13.0<br>Ov., ..... | Exc. | Bouin | C-P | Trans. | 10 | H.-E. | 1943, J. A. York, Baltimore<br>Operative |
| 8118.. | E., 12.6<br>Ov., ..... | Exc. | Bouin | C-P | Frontal | 10 | H.-E. | 1943, W. E. Studdiford, New York<br>In middle third of group; cytology excellent |
| 8253.. | E., 11.2<br>Ov., 30 × 20 × 10 | Good | Bouin | C-P | Frontal | 10 | Al. coch. phloxine | 1944, L. R. Wharton, Baltimore<br>Operative; youngest in group |

The photographs on this plate were selected as showing in faithful detail the external form of a younger member (no. 8253) and an older member (no. 8101) of age group xvii. Between them they represent a range of about two days of development.

Among the most constant surface features are the following characters: The lateral nasal wing now conceals the olfactory invagination in profile views. The auricular hillocks on the mandibular bar have smoothed out into the crus and tragus of the external ear. The hillocks on the hyoid bar still have their embryonic form and constitute a three-hillock plate in contrast with the smoothed-out hillocks in front of the hyomandibular groove. The third pharyngeal bar has disappeared from view. The hand plate is marked with finger rays, very distinct in older specimens. There is a rounded foot plate and in older specimens an indication of the great toe, but otherwise no toe rays.

FIGS. 12–15. No. 8253, ×6.

FIGS. 16–19. No. 8101, ×6.


19
15
18
14
17
13
16
12

As was done in the preceding two horizons, there is shown on this plate a group of photographs illustrating the variation in form encountered in a two-day period of development, in this case, age group xvii.

In spite of variation in fixation, shrinkage, and original condition of the embryo, the external characteristics listed for the preceding plate apply fairly well to this whole group: that is, the lateral nasal wing, the prominence of the three-hillock plate back of the hyomandibular groove, the disappearance of the third pharyngeal bar, the finger rays of the hand plate, and the absence of toe rays except for the prominence of the great toe.

The embryo numbers and enlargements are as follows:

FIG. 20. No. 6742, ×5.
FIG. 21. No. 6519, ×6.
FIG. 22. No. 8118, ×5.
FIG. 23. No. 6631, ×5.
FIG. 24. No. 1267A, ×5.
FIG. 25. No. 6521, ×5.
FIG. 26. No. 6258, ×5.
FIG. 27. No. 6520, ×5.
FIG. 28. No. 5893, ×5.

20

21

22

23

24

25

26

27

28

## Plate 3, xvii

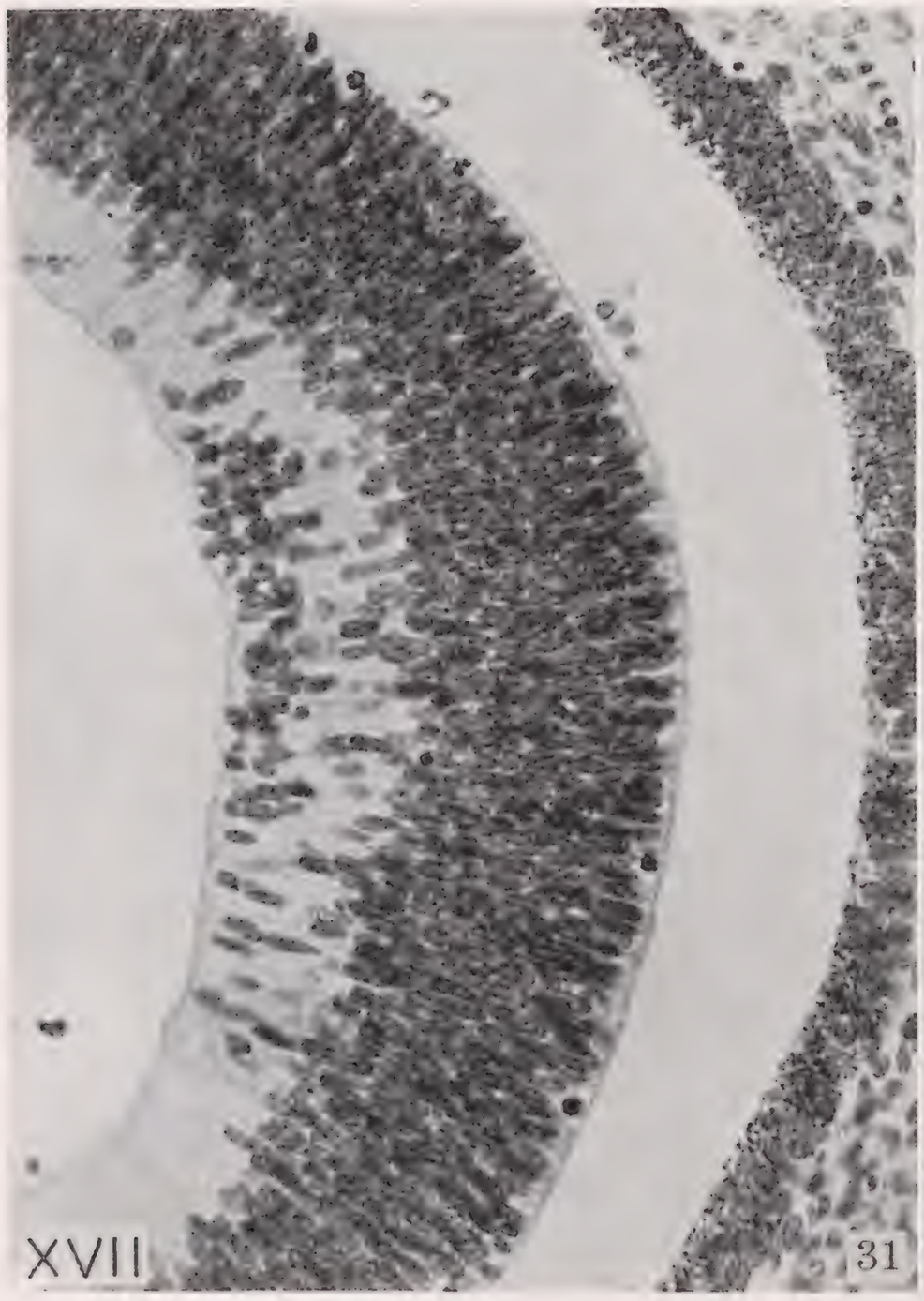
On this plate are shown the characteristics of the eye, as seen under the microscope, in age groups xvi and xvii. Specimens typical of horizon xvi are shown in figures 29 and 30. At that time the lens vesicle has a large lumen. One can see rather sharply marked off the section of its wall that is to become the lens disk, as distinct from the epithelium that is to remain as the thin epithelial layer over the front of the lens in the mature stages. In horizon xvii (fig. 32) the lens disk, in the histogenesis of lens fibers, becomes much thicker and encroaches upon the lens cavity, reducing it to a crescentic shape. Another decisive advance is the migration, out of the primary nuclear layer of the retina, of the cells that are to form the inner nuclear layer of the retina as seen in figure 31. The starting point of this migration occurs at about the location of the future macula lutea. In the next age group it spreads much wider.

The embryos, section numbers, and magnifications are as follows:

Fig. 29. No. 6509, 12-1-3, ×150.
Fig. 30. No. 6507, 7-2-3, ×150.
Fig. 31. No. 6520, 22-2-3, ×300.
Fig. 32. No. 6521, 24-3-4, ×150.

XVI
29

XVI
30


XVII
31

XVII
32

## HORIZON XVIII

### External Form

From external form alone one cannot always distinguish between younger specimens of horizon xviii and older ones of horizon xvii. In drawing an arbitrary border line between the two age groups, as has been done in this study, one must rely on selected structural characters of the inner organs that are revealed in sections. As a consequence, in several instances, the author has found it necessary to change the placement of certain embryos from a provisional one based on their external form to one in the next older or younger group based on the study of the same embryos after they had been cut in serial sections.

To gauge accurately the level of development of an embryo, one must know what it is like inside. There are many things, however, to be learned from the external form. One can see, for instance, that the group of embryos shown on plate 2, xviii is older than the similar group of the preceding horizon which was shown on plate 2, xvii. In addition to being a little larger, it will be seen that the embryo of horizon xviii has made an advance in the coalescence of its body regions, which have now become more closely integrated in a common cuboidal bulk, instead of consisting of individual and obviously separate parts, as was true of earlier horizons.

Other and more particular items that are characteristic of this age group can be identified in the details of various surface regions. Above and below the eye one sees in older specimens of the group the early rudiments of the eyelids and the grooves initiating the conjunctival sacs. The thin outer lamina of the retina now contains considerable pigment, causing the retinal rim to be conspicuous. This is particularly true in formalin-preserved specimens, whose tissues are somewhat transparent. In embryos of horizon xviii this pigmented rim of the optic cup is characteristically quadrangular, owing in part to the forward projection of the upper margin of the retina and in part to the cleft in the lower part of the cup. At the same time, white opaque zones of mesenchymal condensation spread progressively forward over the pigmented area, especially above and lateral to it. These opaque thickenings mark the laying down of the sclera and its muscle sites.

In profile views of the nasal region one can begin to recognize the tip of the nose and the frontonasal angle, from which point the future bridge of the nose is to extend forward. To see the face satisfactorily one must have decapitated heads, or lacking these one can substitute enlarged reconstructions of the face region made from serial sections. Such a model was made of a representative embryo of horizon xviii and is illustrated in the accompanying figure 1, xviii. One can clearly identify the nose with its nostrils, nasal tip or apex, nasal wings, and nasal septum (columella nasi). The associated structures are indicated in the figure. The nose at this time is to be thought of as a raised window awning; later, as the bridge of the nose forms, the awning will be let down. What is now in a vertical plane will then be horizontal. The upper lip of the mouth is not differentiated as a separate structure. It will be formed jointly and in segments by the premaxillary and maxillary centers of the two sides. In this same figure one can see the auricular hillocks, though they are seen better in profile views (pls. 1 and 2, xviii). These hillocks were prominent elevations in the preceding horizon. Here they are merging with one another and the adjacent surfaces, and thus forming the primordia of definite parts of the auricle. Those in front of the auditory cleft are more advanced than those back of it. Thus the upper two hillocks (nos. 2 and 3) of the mandibular bar are losing their individuality in the process of fusing to form the crus helicis. In older specimens of the group, the two upper hillocks (nos. 4 and 5) posterior to the cleft have similarly merged to become the helix. Hillocks 1 and 6 persist and become, respectively, the tragus and antitragus, with the lobulus pendent from the latter.

Turning to the extremities, the hands in all specimens have distinct finger rays with interdigital notches on their rims. In members of the older half of the group one can begin to speak of an elbow. The feet, in some specimens, exhibit toe rays, but their rim is not definitely notched. It is in this horizon that mam-

mary nipples make their appearance just under the arm, near the elbow region. Also, even in some of the older members one can still find a trace of somite

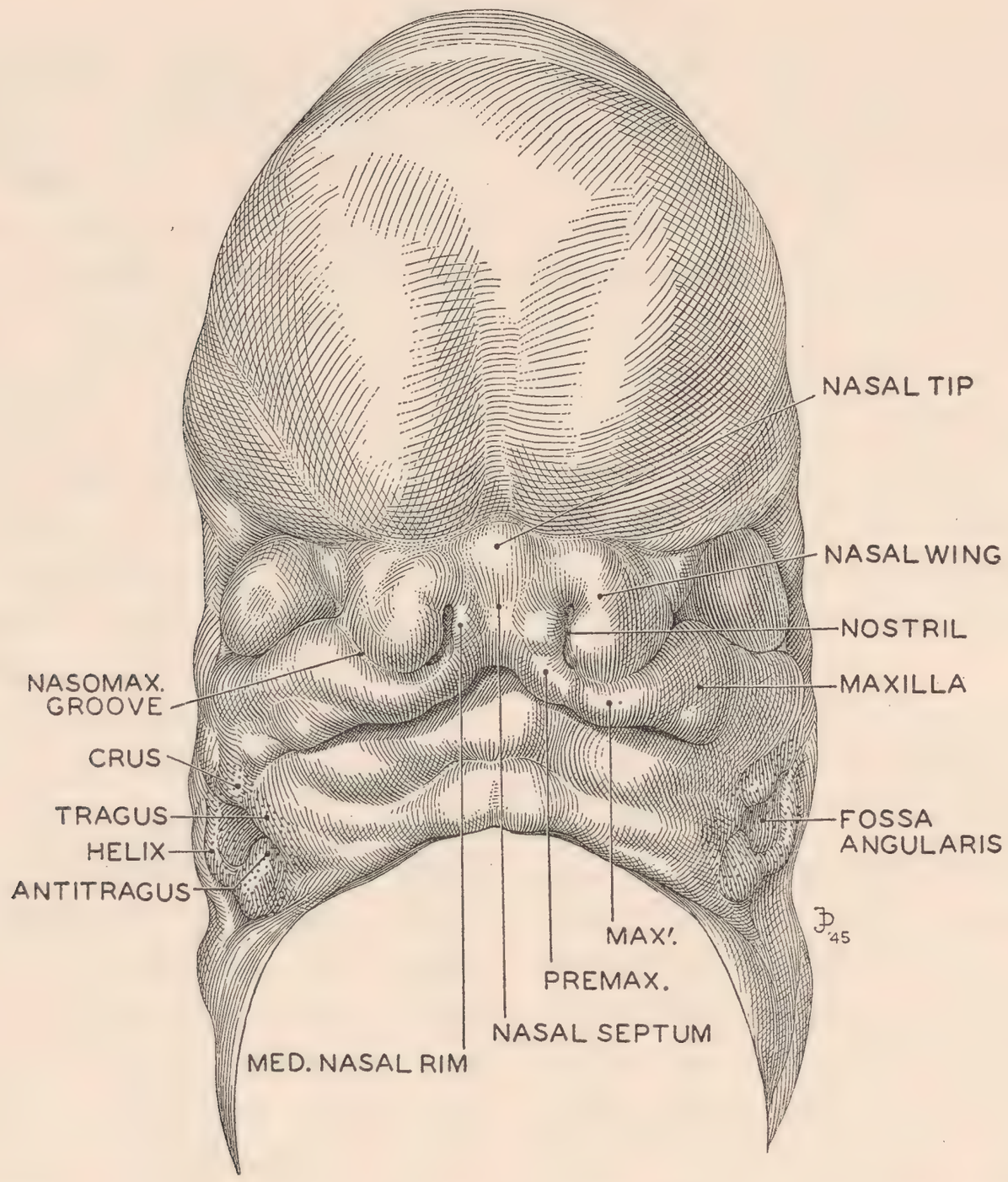



Fig. 1, xviii. Three-dimensional reconstruction of the face of embryo no. 492, a typical representative of horizon xviii. By this time the nose and upper jaw are definitely outlined. There is still a small gap between the right and left upper jaws, which will be smoothed out by the further enlargement and blending of the two premaxillary centers. When that occurs the nasal septum will be closed off from the mouth orifice. It is still too early to speak of lips. The growth center labeled MAX′. is a subdivision of the maxillary center and is distinct from the lateral nasal process (NASAL WING). Between the two is the nasomaxillary groove, from the floor of which an ingrowth of epithelium arises, forming an irregular epithelial flange which is finally transformed into a duct communicating with the nasal passage, the nasolacrimal duct. This figure forms one of a series with figures 1, xv; 2, xvi; and 1, xvii. They are all enlarged 12.5 times and are directly comparable. Drawing made by J. F. Didusch. Reconstruction made by O. O. Heard.

elevations in the lumbosacral region. Rather than be satisfied with what little has been said here, it is hoped that the reader will examine carefully the photographs on plates 1 and 2, xviii. All the embryos illustrated have been serially sectioned and studied on the slide, and all of them conform microscopically to the criteria arbitrarily set for horizon xviii. The two embryos on plate 1, xviii are type specimens taken respectively from among younger and older members of the age group. The embryos on plate 2, xviii illustrate such variations as are incident to differences in photography, methods of fixation, and variations in the degrees of development encountered within this horizon. The most advanced specimen (fig. 27) is on the border line of the succeeding age group.

## Size and Age

In considering the size of embryos in horizon xviii we need to distinguish between its older and younger members. Inasmuch as the Müllerian duct is present in all the specimens of this age group, being quite short in the younger specimens and progressively longer as growth advances, it can be used as an index

of general development. Taking advantage of this fact, the 35 embryos were arranged in two nearly equal-sized groups, a younger one (19 specimens) in which the length of the Müllerian duct is 0.6 mm. or less, and an older one (16 specimens) in which it is more than 0.6 mm. In the younger group there are 3 embryos which are exceptionally small, less than 13 mm. The other 16 specimens range from 13.0 to 16.5 mm., with an average greatest length of 14.6 mm. In the older group, if we disregard 2 exceptionally small embryos and 1 exceptionally large one, there remain 13 embryos whose greatest length ranges from 14.0 to 17.2 mm., with an average of 16 mm. In fact, 7 of the 13 specimens range between 16.0 mm. and 16.8 mm. It follows that embryos belonging in the younger half of this horizon may be expected to have a greatest length of about 14.5 mm., and those in the older half will in most cases be about 16 mm. long.

If one takes the group as a whole and disregards criteria as to the status of development of the individual members, it is found that a decided majority (61 per cent) range from 14 to 16 mm. in greatest length. This then may serve as the expected length for the horizon. If it is necessary to know the range of variability, the reader is referred to the graph shown on page 136. It is to be remembered, however, that the scattering found there is due in part to extraneous factors.

The available records of the size of the chorion are not so numerous for this age group as one could wish. The embryos are now large enough to be easily handled by physicians untrained in embryology, and it is common practice among them to save the embryo only. Out of the 34 specimens comprising the group, the size of the chorion was recorded in 14. Of these, 3 were tubal specimens, which are invariably undersized; 1 was abnormal owing to a fibroid uterus; 1 was macerated, and 1 was complicated by a twin pregnancy. This leaves 8 specimens having usable records. In 7 of these (85 per cent) the largest diameter ranged from 40 to 51 mm. The eighth had a largest diameter of 38 mm., but in this case the villi were defective. It is to be added that all three principal diameters of these chorions were about equally large. In each of the 8 specimens the total of the three principal diameters was 100 mm. or more, the largest being 120 mm. In the preceding age group more than one-half of the 9 adequately recorded chorions had a three-diameter total of less than 100 mm., and the largest of those above this had a total of 108 mm. If these figures were converted into terms of volume, it would be seen that the volumetric increase in the size of the chorion of horizon xviii over that of xvii is considerable.

When the Carnegie laboratory assembled its collection of macaque embryos, attention was centered on the earlier stages, and in this study we have now come to levels where macaque specimens of known ovulation age are more and more widely separated. For horizon xviii there is but one matching macaque embryo. This is no. C 658, which has an ovulation age of 36 days.[1] This macaque embryo falls somewhere between embryos nos. 8097 and 7707, which are herewith illustrated on plate 1, xviii. The matching is based on such characters of external form as notched finger rays, toe rays barely perceptible, auricular hillocks transforming into definitive parts of the auricle, heavy retinal pigmentation, white condensations overlapping the retina laterally and above, and lastly the beginning eyelid folds. This embryo has not been sectioned, which precludes additional evidence from its internal structure. From the well marked characters of its external form, however, we are justified in assuming that it is in an equivalent state of development to the two human embryos above mentioned, and we may further assume that the human embryos have about the same ovulation age as the macaque embryo, namely 36 days. It is to be noted that the macaque embryo is 13 mm. long, whereas the greatest length of the two human embryos which it so closely resembles is 15.5 and 14.5 mm. respectively. Whether it is generally true that after the first month macaque embryos are smaller or larger than human embryos of equivalent development is a question which must await the evidence of an adequate number of macaque specimens.[2] We are already seeing in this study, however, that variation in size of embryos depends on fortuitous factors that render

[1] C. H. Heuser and G. L. Streeter, Development of the macaque embryo, Carnegie Inst. Wash. Pub. 525, Contrib. to Embryol., vol. 29, pp. 15–55, see table p. 54, pl. 32, 1941.

[2] Compare A. H. Schultz, Fetal growth and development in the rhesus monkey, Carnegie Inst. Wash. Pub. 479, Contrib. to Embryol., vol. 26, pp. 71–97, 1937.

size a less dependable index of age than characters revealing developmental levels.

The characteristics of the external form, size, and age of embryos included within horizon xviii can be summarized as follows: (1) Because of the surface blending of its parts and its increased thickness (width), the body of the embryo is a more unified cuboidal mass than in earlier groups, and in all the specimens there is some indication of both cervical and lumbar flexures. (2) The extremities are somewhat longer; the digital rays of the hand are definitely notched; the elbow region is usually discernible, and on the foot plate the toe rays can be seen in some specimens. (3) Eyelid folds are present in the older specimens, and the pigmented retina is partly covered by white opaque condensed scleral masses, particularly above and on the lateral side of the retina. (4) A distinct tip of the nose can be seen in profile. (5) Auricular hillocks are being transformed into definite parts of the external ear.

As for size, the embryos in the younger half of the group are commonly about 14.5 mm. in length, and those in the older half about 16 mm., several being 17 mm. long. Taking this group of embryos as a whole, the majority of them fall within the range of 14 to 16 mm. greatest length. In the 8 usable cases where the size of the chorion was recorded, the three-diameter total was in each instance 100 mm. or more, the largest being 120 mm. Comparing these embryos with macaque specimens of known ovulation age, the estimated age of embryos belonging in horizon xviii is placed at $37 \pm 1$ days.

## Organogenesis

By way of introduction, before entering on a survey of the inner organs, it is recommended that the reader examine figure 10, xviii, which shows certain easily definable structural features. These are present throughout the group and compose a syndrome of characters which, under the arbitrary procedure adopted in this study, determine whether or not a given embryo qualifies as a member of this horizon. As we proceed with the detailed microscopic examination of the various systems, we shall presently see that many other characters fit in with this syndromic group, in addition to the few simple ones shown in the figure mentioned.

### *Nasal Passages*

As part of the nose (fig. 1, xviii) we have already had a surface view of the nostrils. We remember them as the rims of the right and left olfactory depressions, that is, lines of junction of placodal ectoderm and skin ectoderm. The depressed center of each placode takes the form of a deep pocket, and, owing to the growth of its own epithelium and the enveloping effect of the proliferating surrounding tissues, it becomes a supplementary air passage. This passage at first ends blindly, but in the older members of this age group its blind end opens through what had been the temporary bucconasal membrane and participates in the formation of the funnel-like transition into the pharynx. A communication between the respiratory tract and the surface of the face is in this way provided that by-passes the mouth, leaving the latter free to perform its own special functions.

Transverse serial sections through the nasal passages of two embryos belonging in this age group, one slightly younger than the other, are shown in figure 2, xviii. In the younger one (no. 6524) it can already be seen that the walls of the passage are differentiated into regions, and that the ethmoidal epithelium (upper part of median wall) is becoming distinct from the maxillary territory of the lateral wall. The shallow fold in the lower part of the medial wall marks the vomeronasal organ (Jacobson's). These regional demarcations are still more evident in the older embryo (no. 4430). The precocity in differentiation of the epithelium entitles the latter to high rank as a determining factor in the form regulation of this region. It performs in the same manner as gut epithelium. Both of them are characterized by mosaic-like organ-forming regions, whose boundaries are evident very early.

In these serial sections one can trace the fate of the nasal fin. We have already seen it, in horizon xvii, as a plate of epithelium that maintains a connection between the nasal placodal epithelium and the ordinary surface epithelium, in line with the groove be-

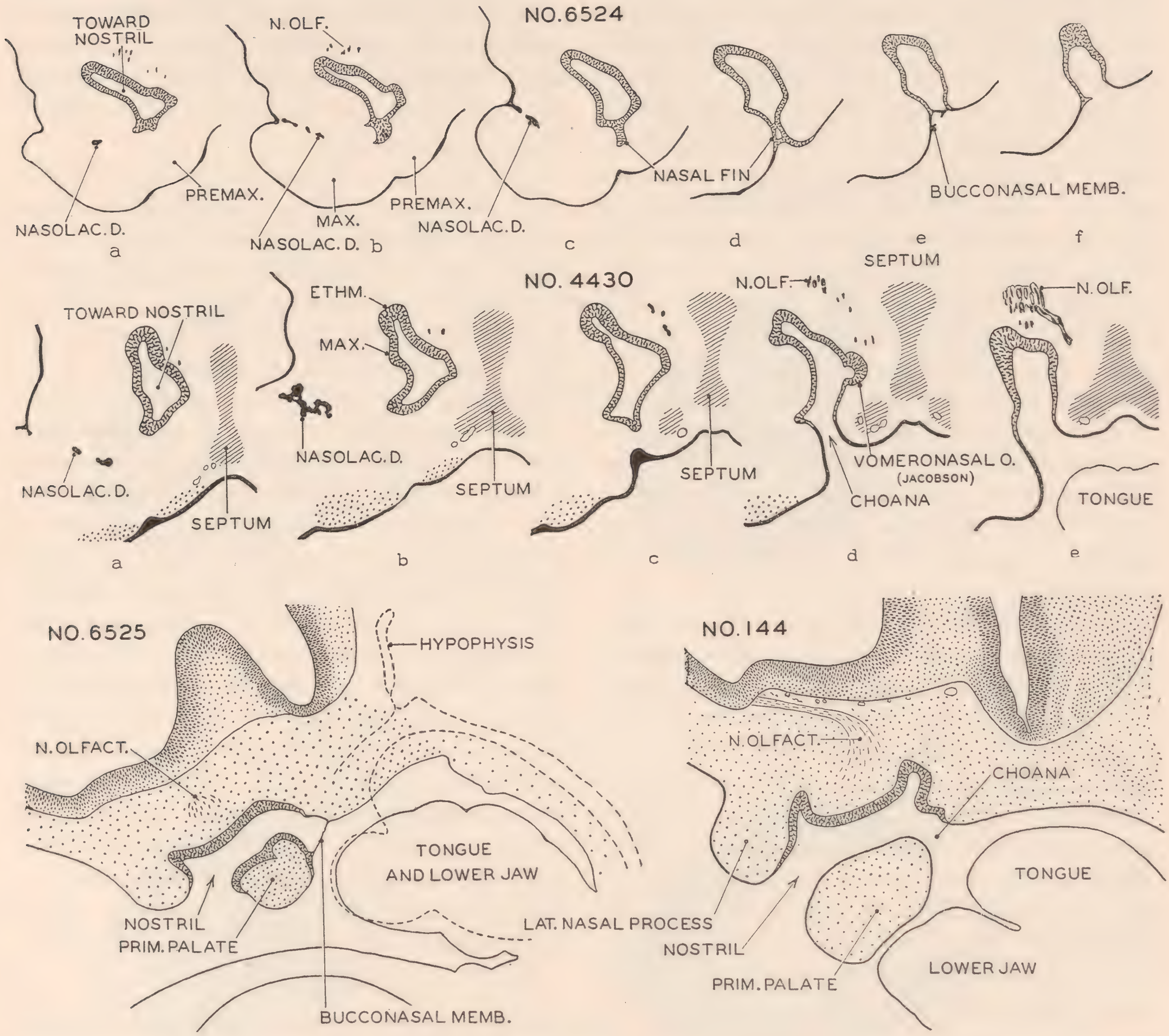



FIG. 2, xviii. Nasal passages in embryos of horizon xviii. Upper row shows transverse sections, at equal intervals, through right nasal passage of a younger member of the age group (no. 6524). Section *a* is the most rostral and *f* is the most caudal. In the caudal levels (*d–f*) the nasal epithelium, by means of its finlike growth, has retained its continuity with the surface (oral) epithelium. Instead of remaining a solid plate, the nasal fin is now splitting apart to form the choana, its last remnants producing the bucconasal membrane.

The middle row (embryo no. 4430) represents an older member of the age group, and the nasal epithelium shows distinct specialized regions. The ethmoidal field is distinct from the maxillary field, and the choanal field differs from both of these. Also there is a definite vomeronasal organ (section *d*). An irregular strand of epithelium sprouting from the under surface of the nasomaxillary groove is growing toward the nasal epithelium and will constitute the nasolacrimal duct (*a* and *b*). The beginning of this could already be seen in the younger specimen, no. 6524. A mesenchymal condensation now marks the primordium of the median cartilaginous septum of the nose.

The two parasagittal sections of the bottom row show the final step in the establishment of a through nasal passage (the respiratory by-pass). On the drawing of no. 6525 is superimposed the outline of a median section, shown by broken line, showing the relative position of the tongue. The drawing of no. 144, an older embryo, is a composite of two consecutive sections. All drawings in this figure ×25.

tween the maxillary and premaxillary growth centers. In transverse sections the nasal fin has the appearance of a ventral stem to the nasal passage. In the present age group, in the more rostral sections, it becomes disconnected and is taken up by the overlying nasal epithelium. Caudally, this fin cleaves open to form part of the posterior nares. In doing so it becomes stretched to form the bucconasal membrane, and this in turn is detached and absorbed in the wall of the choana, leaving a free respiratory passage. The topographical relations of the nasal passage are further simplified by sections made parallel to the sagittal plane. Two embryos thus sectioned, a younger one (no. 6525) and an older one (no. 144), are also shown in figure 2, xviii. Although in the older of these two the respiratory by-pass is an open way, its mature relations are not yet established. These will be attained through differential growth which is accompanied by a relative shifting forward of the tongue and lower jaw.

Note is to be taken of the initiation of the nasolacrimal duct, an event that can be advantageously studied in embryos belonging in this group. In figure 2, xviii, embryo no. 6524 *b* and *c,* and embryo no. 4430 *a* and *b,* is shown an irregular lamina or strand of epithelium sprouting from the under surface of the nasomaxillary groove. This epithelial strand makes its way downward through the mesenchyme, eventually to join the lateral wall of the inferior meatus of the nasal passage. The transformation of this epithelial strand into a duct and the junction of its upper end by two small canals with the conjunctival sac occurs later in fetal life.

### *Lung*

The pattern of the bronchial tree is shown in outline in figure 10, xviii, as one of the syndrome of characters of this age group. The right and left stem bronchi with their bulbous terminals continue to dominate the pattern. They have primary and secondary branches and the secondary branches are now further bifurcating. The structure of the trachea was shown in the oldest of the series of stages illustrated on plate 3, xv. An examination of this photograph (embryo no. 4430) reveals that the tissues surrounding the tracheal epithelium have made definite advance in differentiation. It will be recalled that this is visceral mesenchyme, derived for the most part from proliferated migrants from the surface cells of the coelomic channel. The survivors of the latter in the thoracic region have now flattened down into a relatively inactive mesothelium and are destined, according to their location, to form the pleural sacs and pericardium.

It has been seen as far back as horizon xii, with the first emergence of the lung diverticulum, that there is a slight difference in appearance between the epithelium that is to become the primary bronchi and that which is to become the esophagus. Transverse sections through the esophagus and trachea show slight differences both in the epithelium and in the surrounding mesenchymal condensation in horizon xv, and characteristic increases in these differences mark the two-day advances between the succeeding horizons sufficiently for their identification. Particularly is this so for the present horizon xviii. Still referring to plate 3, xv, embryo no. 4430, one finds a marked difference between the trachea and esophagus, both in their epithelium and in the supporting walls. The esophagus is now characterized by the primordium of its muscle coat, between which and its epithelium is the light zone of embryonic reticulum. In contrast, in the large-lumened trachea the primordium of the cartilage ring is still directly apposed to the epithelium. Elsewhere one sees the thick-walled pulmonary arteries and still thicker thoracic aorta. The very large right vagus trunk is enclosed in a thin but definite connective-tissue sheath. Only a small part of the left vagus is shown in the picture.

### *Heart*

In this age group the heart approximates its mature fetal equipment as a composite, four-chambered organ with separation of the pulmonary and aortic blood streams. In the older members of the group the crest of the interventricular septum has fused with the loose tissue of the opposing wall of the primary heart tube, producing the septum membranaceum and closing off the cavity of the left ventricle from that of the right.[3] The aortic and pulmonary valves could already be recognized in the preceding horizon. Here they have acquired increased definition and are becoming cup-shaped. The tricuspid and mitral valves are later in their differentiation, but one can now

[3] In this connection the reader is referred to the studies of P. N. B. Odgers, which appeared in 1935, 1938, and 1939, in the Journal of Anatomy (Cambridge, England).

recognize the ridges of condensed gelatinous reticular tissue that are to form them. Even in this crude form it is probable that they have some valvular function and favor the drive of the right and left blood streams.

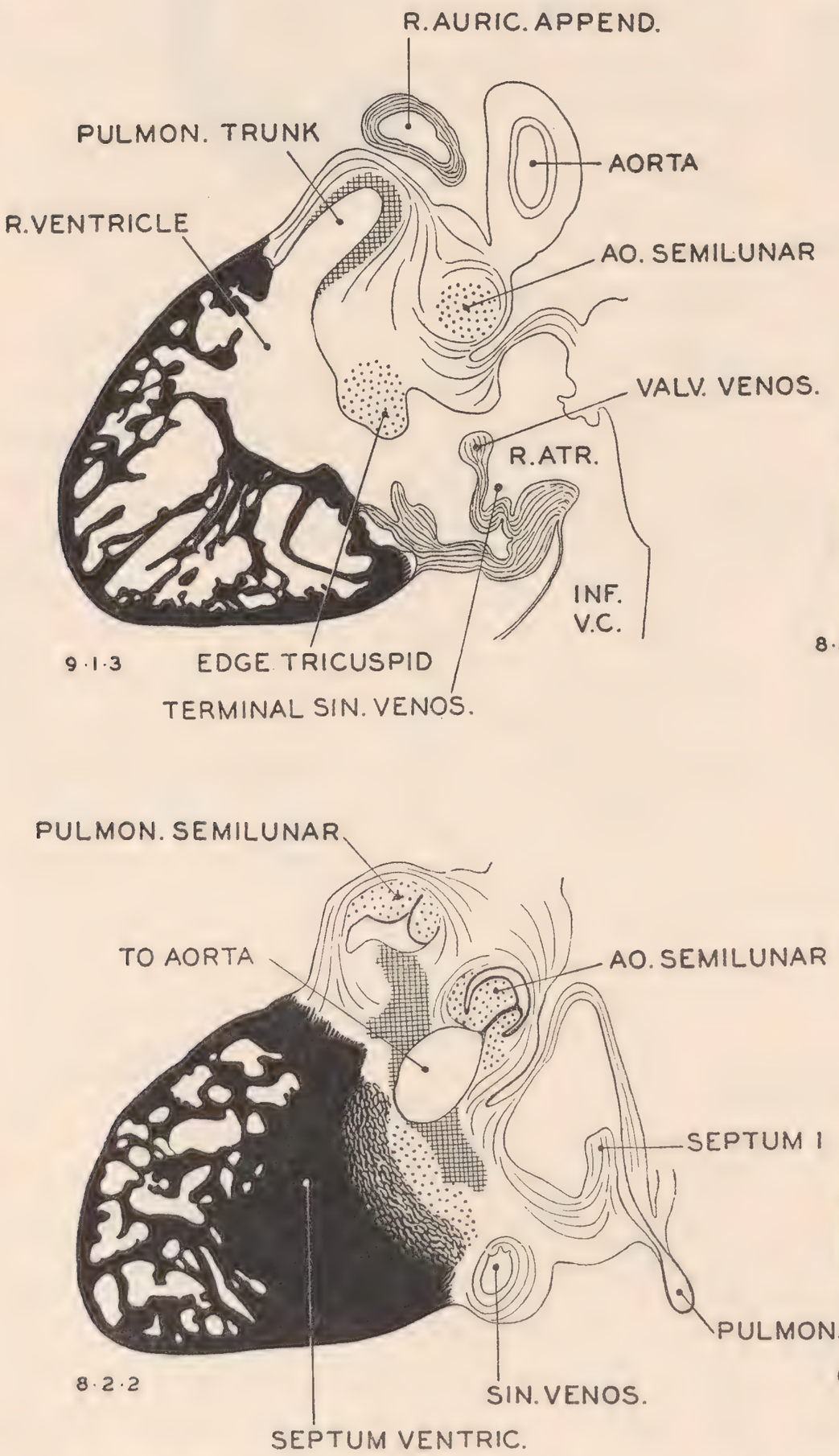



FIG. 3, xviii. Sagittal sections through heart (embryo no. 406) typical of age group xviii. The crest of the ventricular septum has now fused with the loose tissue wall of the primary heart tube, completing the separation of the aortic and pulmonary blood channels. The semilunar valves are now distinctly marked off. The tricuspid and mitral valves are scarcely more than rounded ridges of gelatinous reticular tissue. The latter tissue is last seen in these valves. ×30.

Drawings of selected sagittal sections of a typical heart from one of the older members of the group are shown in figure 3, xviii. Here in simplified form one can see the composite nature of the heart and the communications and relations of the three kinds of structure involved. It is hoped that the labeling will make a systematic description unnecessary. It may be pointed out, however, that in the condensing gelatinous reticulum of the primary heart tube one sees, in such sections, sheets or areas of more condensed tissue, shown in the figure by hatched lines, which underlie the endothelium and mark the proximity of cleavage and of wall adjustment.

From a frontal series of sections of another embryo of about the same level of development, a transparent reconstruction was made of the heart. Ink tracings were made, on sheets of cellulose acetate, of each section, properly enlarged and oriented. These were superimposed in a series of five detachable groups or study slabs (A to E), as is illustrated in figure 4, xviii. The location of the front surface of each slab

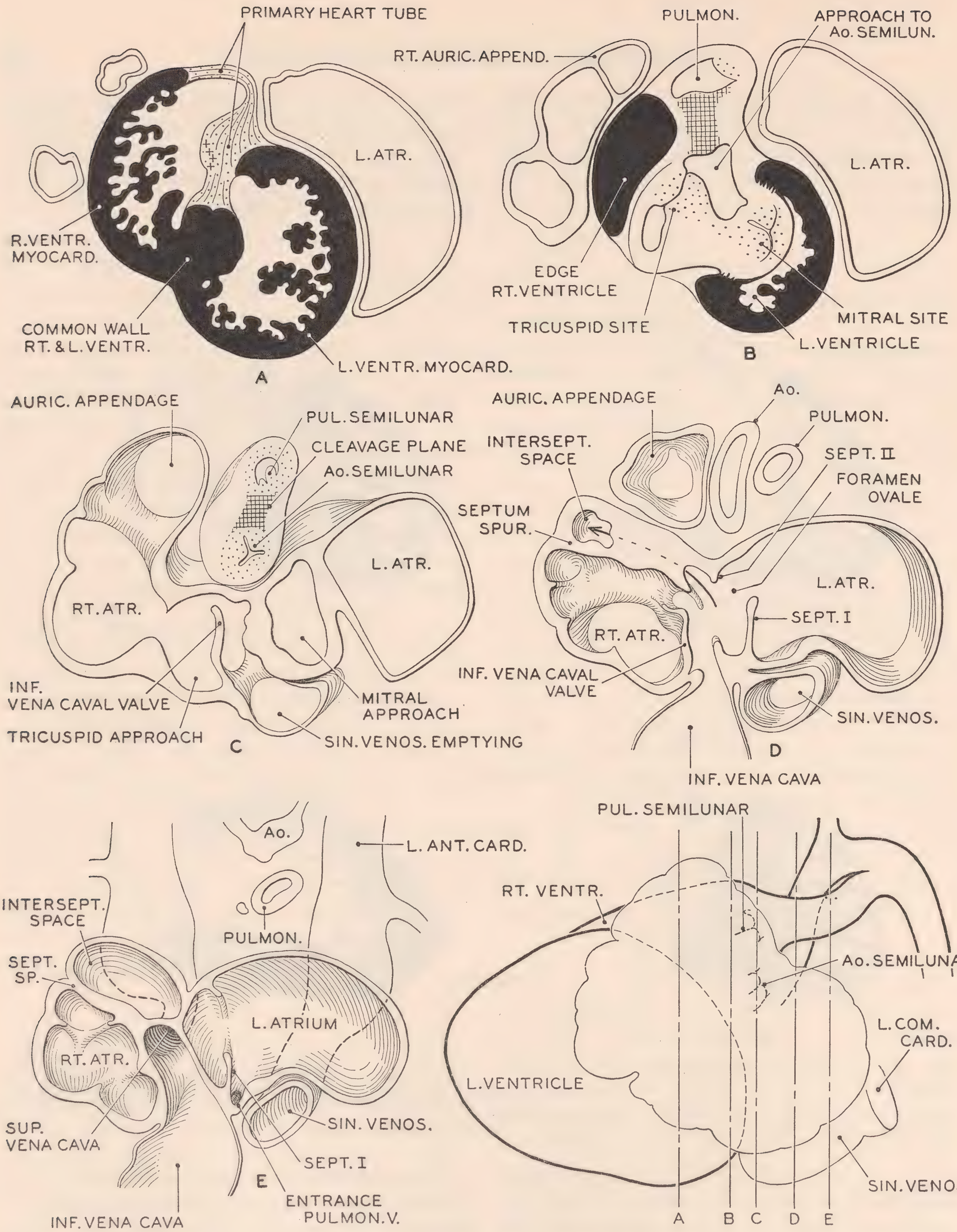



FIG. 4, xviii. Transparent reconstruction of heart (embryo no. 492) typical of age group xviii. In the lower righthand corner the heart is shown in left profile. The vertical lines through it indicate the front surface planes of the five transparent slabs shown separately as A to E. Each slab consists of a series of cellulose acetate sheets, properly oriented and spaced so as to give the correct third dimension. Tentative assembly showed that for reasons of clarity the slabs should not be thicker than is shown in the adopted levels. It will be noted that although the separation of aortic and pulmonary endothelial channels has been effected, the periendothelial coats are still more or less blended from the semilunar valves inward. Peripherally they are separate and have acquired their individual established walls, with no trace left of the gelatinous reticulum. Ventricular myocardium is shown in solid black. Cleavage planes are shown by crossed lines. ×25.

is indicated on the profile view of the heart, shown as an explanatory sketch in the lower right corner of the figure. In this figure, as in the preceding one, the ventricular musculature is shown in solid black to distinguish it as a specialized tissue from the condensed gelatinous reticulum of the primary heart tube and the venous type of muscle wall of the atria. In slab A one sees the nature of the septum membranaceum separating the right and left ventricles. In slab B, directly behind it, is shown the fusion of the wall of the primary heart tube, which thereby separates the tricuspid canal from the aortic corner of the left ventricle. In slab C is shown the complete separation of the aortic and pulmonary trunks so far as the endothelium is concerned. Adjustments, however, are still in progress in the muscle and connective-tissue coats. The tricuspid passage and the mitral passage are the more mature right and left atrioventricular canals. The sinus venosus is shown as it opens into the right atrium, just to the rear of the inferior vena cava. The two freely open, at the same time, through the foramen ovale into the left atrium (slab D). In slab E is shown the manner of entrance of the superior vena cava. It is thus clear that from these three sources (sinus venosus, inferior vena cava, and superior vena cava) practically the whole of the incoming blood now arrives at the entrance of the foramen ovale.

### *Müllerian Duct*

We have already seen several types of specialization of the coelomic epithelium, and attention is now directed to another one. In this case a precise area of the coelomic epithelium, at the cephalic end of the mesonephros, thickens and invaginates to form the female genital duct. This area is regarded by some investigators as a reconversion of unused pronephric epithelium. Without offering any evidence on that point, we shall proceed with the well known fact that the site of its infolding becomes the tubal ostium. This ostium has already become defined before the embryo has arrived at horizon xviii. But it is during this horizon that the invading Müllerian duct makes the principal part of its way down along the front of the mesonephros, as a lateral companion to the mesonephric duct. The size and extent of invasion of the Müllerian duct in an embryo belonging among the older members of the age group is illustrated in the accompanying figure 5, xviii.

The reason for calling attention to the Müllerian duct is that its origin and rate of growth and elongation are quite precisely regulated; so much so that it provides us with an additional definable character for determining the level of development of any given specimen falling within this general period. The duct tends to be straight, and its length is easily calculated in transverse sections by counting the sections and multiplying by their thickness. In sagittal or frontal series the ducts and their environment can be traced with a camera lucida under a suitable magnification, and several such drawings for a given embryo, when superimposed, suffice for making a reconstruction accurate enough for calculating the actual length of the duct. By these devices the length of the more favorable of the two Müllerian ducts was determined for each of the 34 embryos assigned to horizon xviii. It was found that the length of the duct varied in quite accurate accord with the level of development of other parts of the embryo. For example, in embryos where but one semicircular duct (or canal) had been

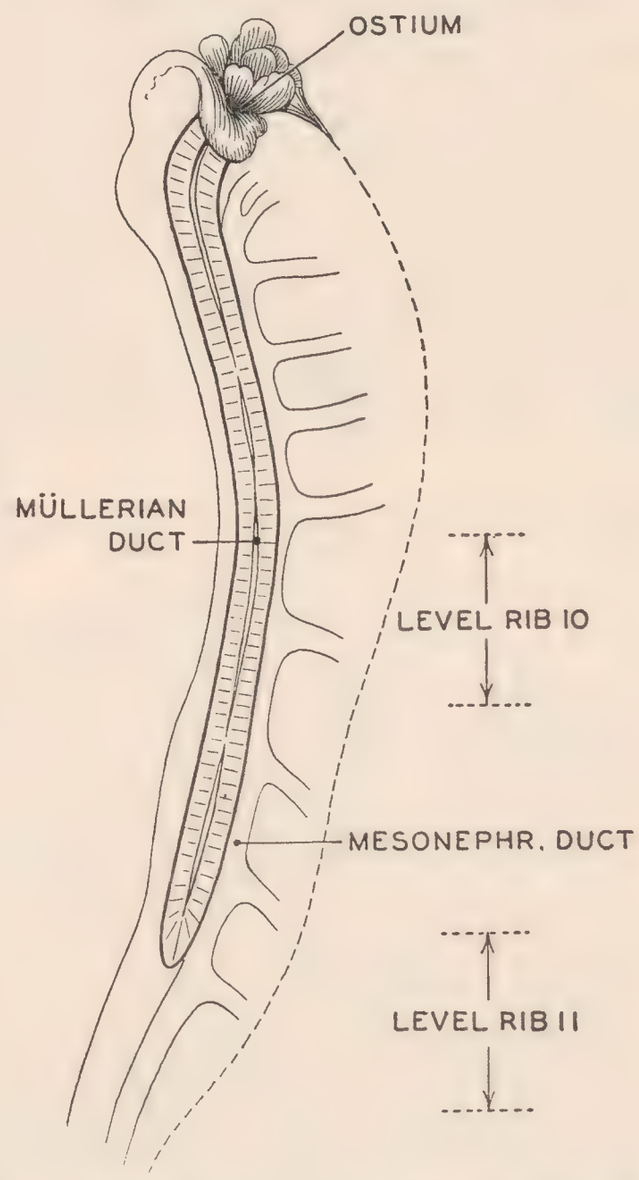



FIG. 5, xviii. Topography of the Müllerian duct in an older specimen (no. 406) of age group xviii. The rib level refers to its free end and can be determined only approximately. Drawing made by J. F. Didusch. ×100.

pinched off in the membranous labyrinth of the ear, the Müllerian duct ranged from 0.2 to 0.4 mm. in length. Where there were two semicircular canals, the Müllerian duct ranged from 0.4 to 0.7 mm. in length. Finally, among the oldest one-third of the age group, where there were three semicircular canals, with a few exceptions the Müllerian duct ranged from 0.8 to 1.1 mm. in length. This relation is expressed in simplified form in figure 6, xviii. It will be seen that from the status of the Müllerian duct one can arrive at the degree of development of an organ as remote and comparatively unrelated as the inner ear. Variations, due to inaccuracies in defining

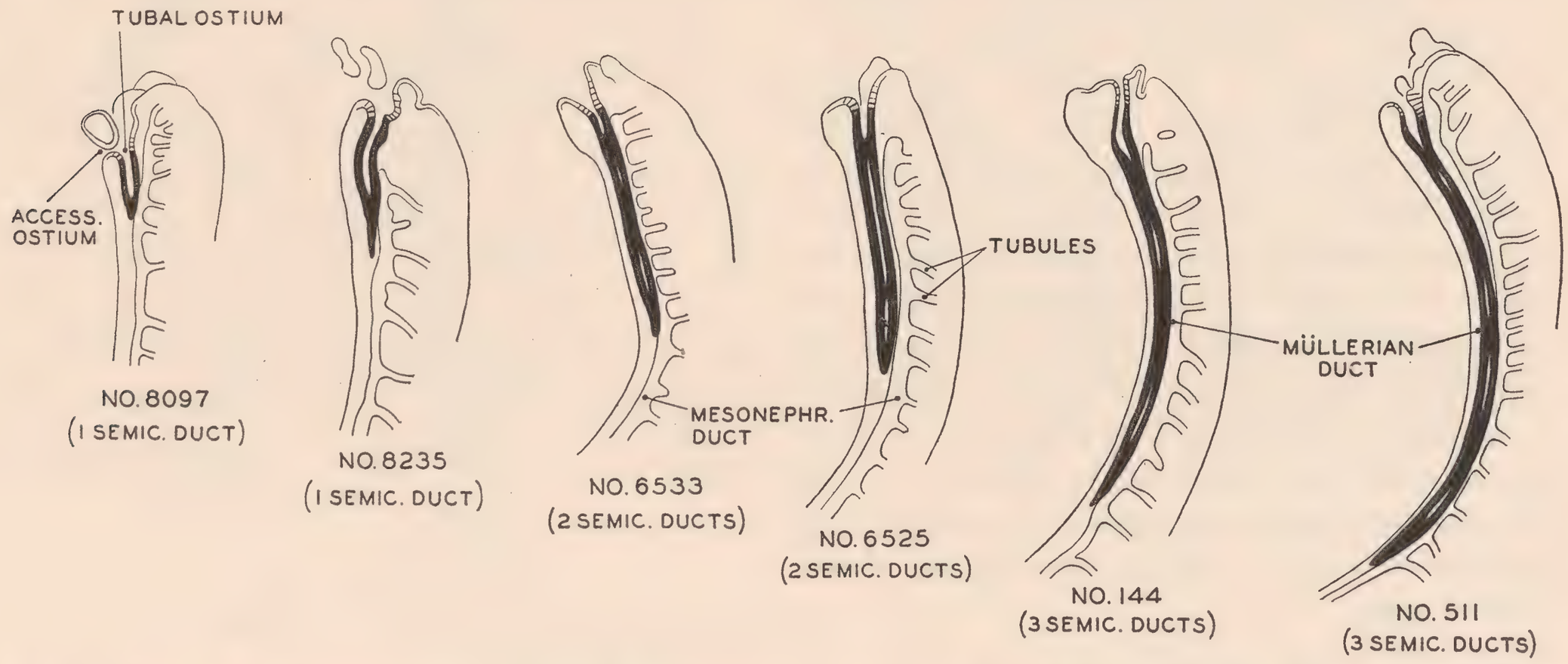



FIG. 6, xviii. The downward extension of the Müllerian duct occurring during age group xviii. Younger specimens are toward the left and older toward the right. Their order is based on the length of the duct, but this is found to correspond closely to other developmental characters. For example, in the younger ones only one semicircular duct is established in the inner ear, whereas the older ones have the full complement of three semicircular ducts. ×50.

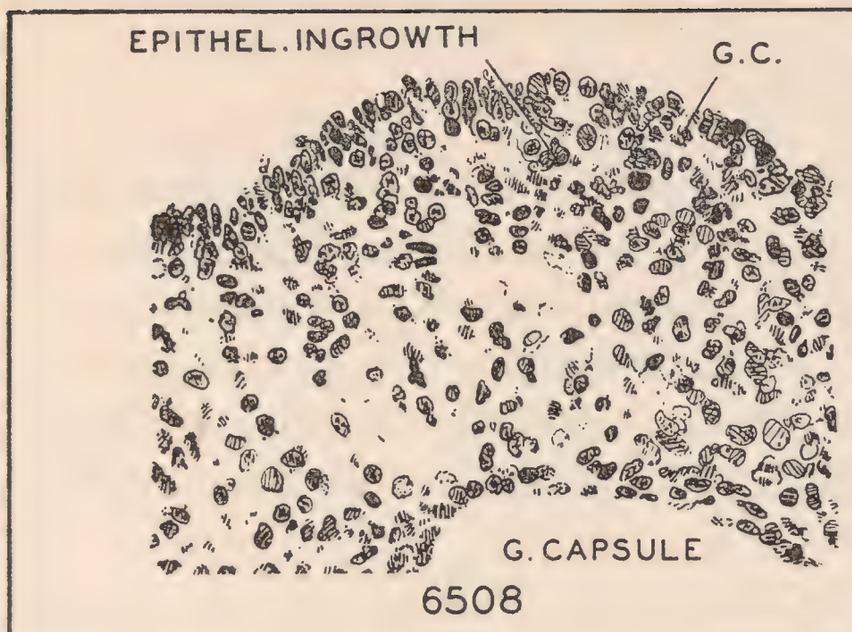
the upper ends of the duct and in making suitable allowance for the fimbriae of the ostium, are not great enough to invalidate the duct length as a useful index of developmental level. Furthermore, the difference that enters as a sex character does not occur until somewhat later than the period we are covering.

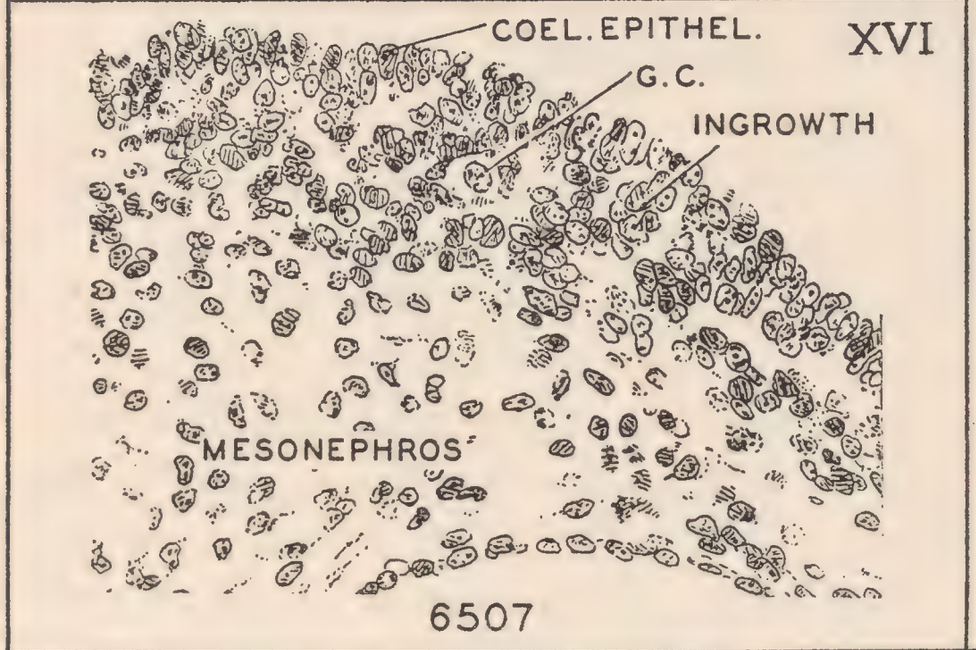
*Gonad*

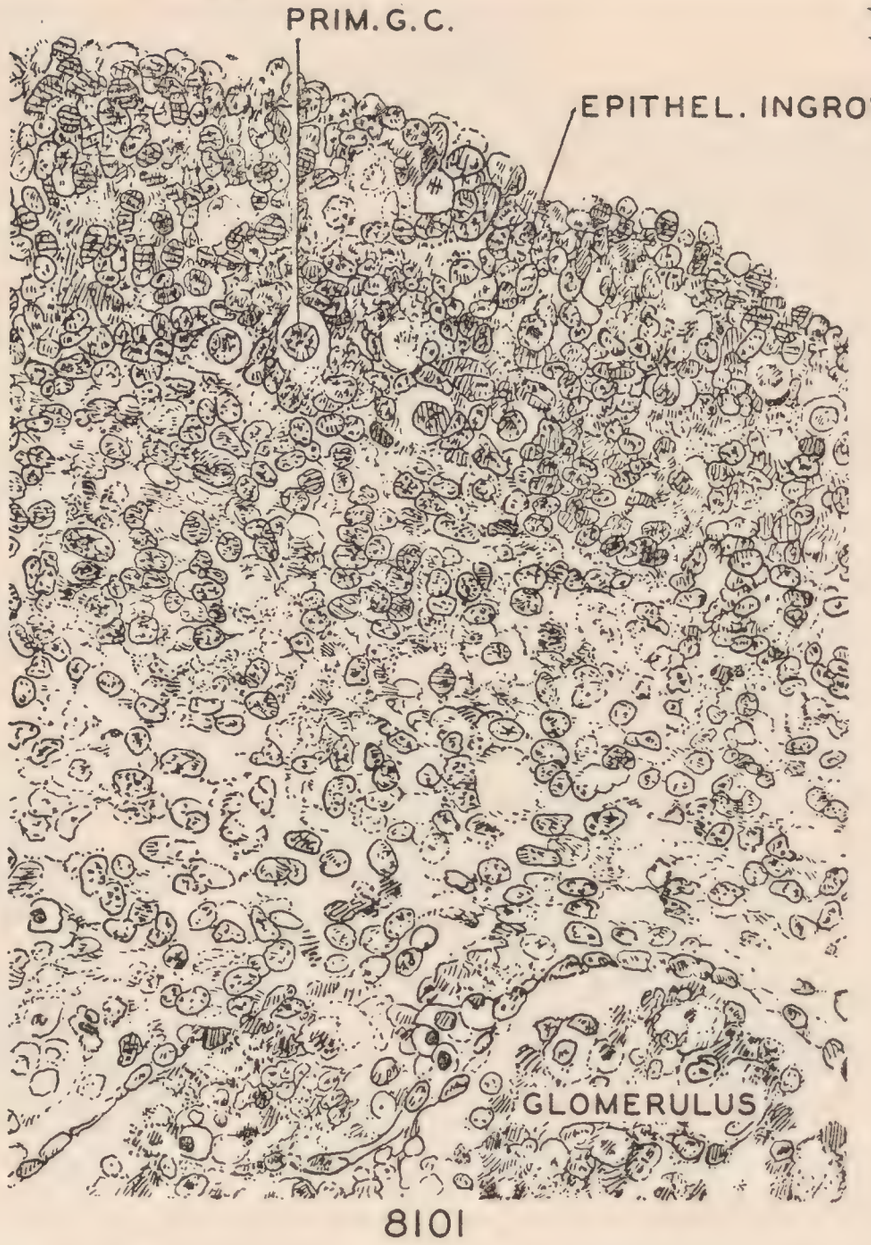
Starting with the original germ disk, one can follow the segregation of the primary embryonic tissues, such as (1) neural ectoderm, (2) skin ectoderm, (3) notochordal process, (4) gut endoderm, and (5) the complex sheet of cells of various kinds and potentials that are included as a group under the term mesenchyme. These stand out in contrast with one another and with the undifferentiated mass of the primitive streak. Throughout the presomite stages the latter is still primitive and represents the remnant of the original unsegregated germ mass which carries on as a proliferating caudal bud. It goes on giving off the primary embryonic tissues and is not exhausted until the total axis system has been laid down. Under horizon xi a description has been given [4] of the coelomic channel that forms through the peripheral part of the complex sheet of mesenchyme. In later horizons it has been seen that the surface cells of these coelomic channels undergo active proliferation, and, in accordance with the area concerned, differentiate into such varied structures as myocardium, framework of liver, connective-tissue coats of the trachea and of the esophagus, and other parts of the gut tract. In the preceding section it was seen that a restricted area of coelomic epithelium produces the highly specialized and important Müllerian duct.

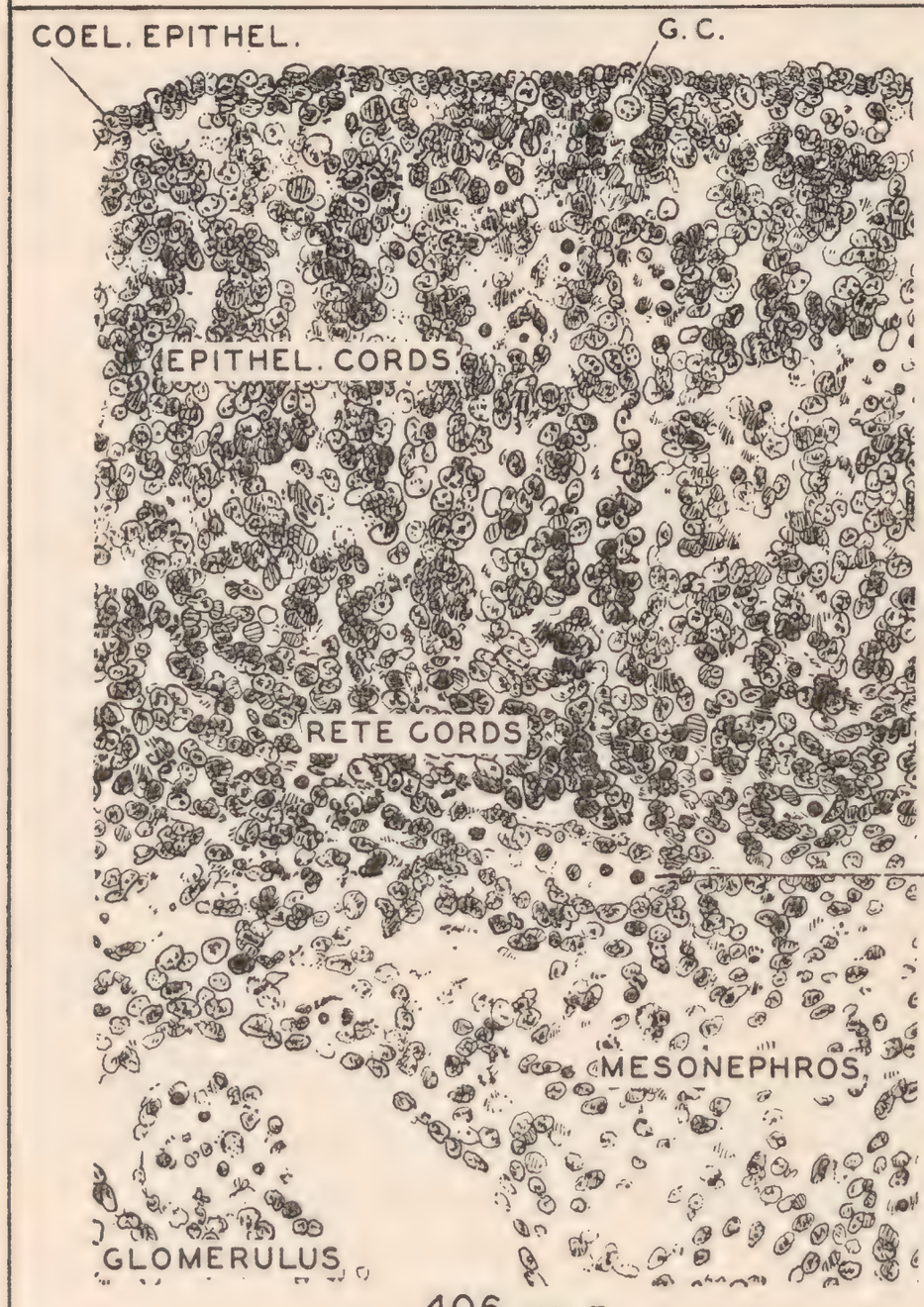
In the light of the above evidence of the marked range in the potentialities of various areas of the coelomic epithelium, one is not surprised to find it playing an essential part in the formation of the gonad. The origin of the primary germ cells and their migration to the surface of the germinal ridge are described

[4] G. L. Streeter, Developmental horizons in human embryos. Description of age group xi, 13 to 20 somites, and age group xii, 21 to 29 somites, Carnegie Inst. Wash. Pub. 541, Contrib. to Embryol., vol. 30, pp. 211–245, see pp. 217–220, 1942.



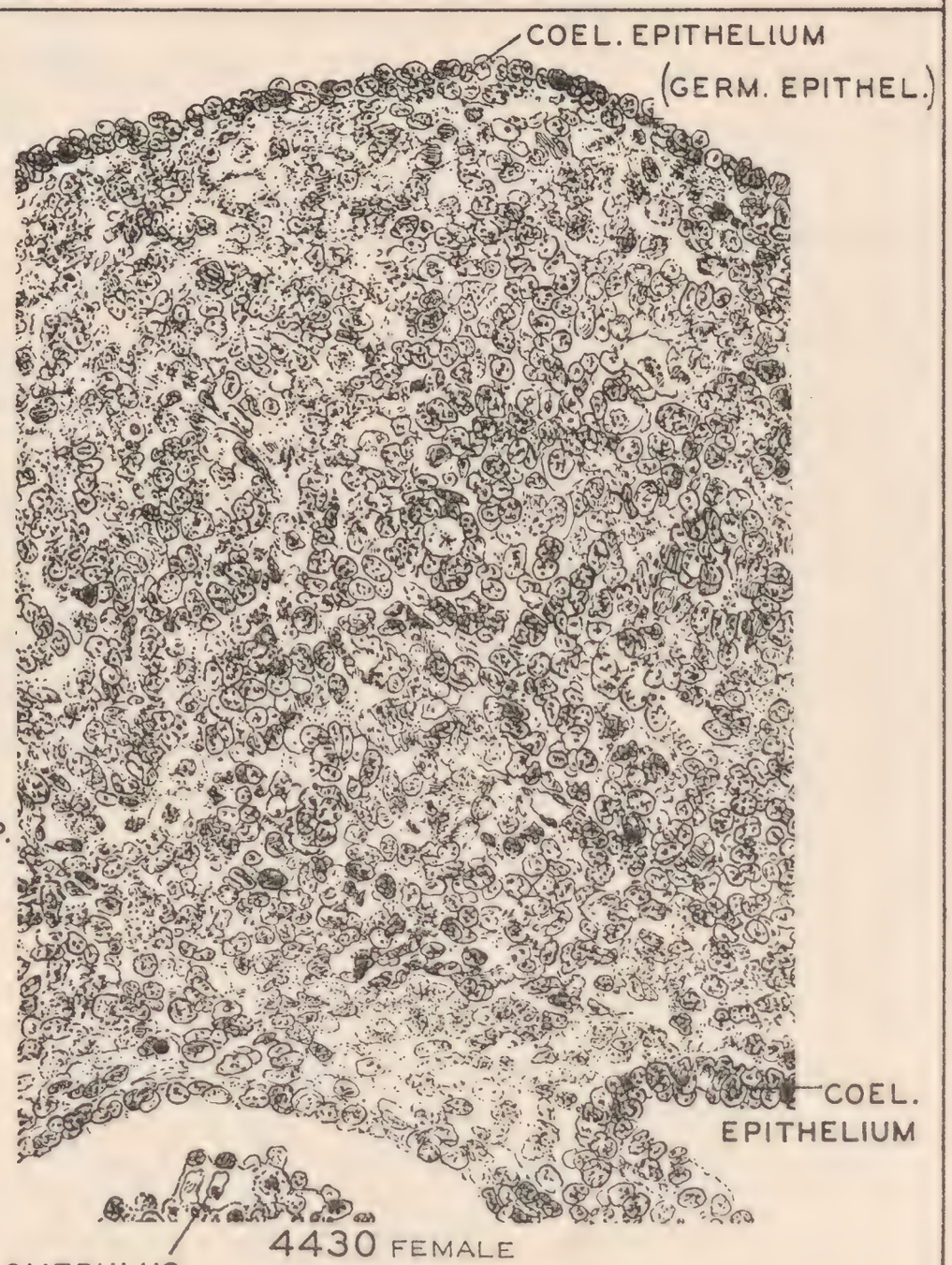




FIG. 7, xviii. Comparable sections passing transversely through the gonad in embryos selected respectively as typical of horizons xv to xviii. Age groups xv, xvi, and xvii show progressive stages of invasion and proliferation of the coelomic epithelium, providing the gonadal framework and follicular clusters around the primary germ cells. Corresponding to the invasion phenomenon, the surface is irregular and one does not find a discrete surface layer of germinal epithelium until age group xviii is reached. It is in this latter group, among its older members, that one begins to recognize sex differences. An example is shown of the male type of epithelial cords with active angiogenesis (embryo no. 406) and of a contrasting female type (embryo no. 4430). In both of these the surface of the gonad is smooth, and there is a distinctly marked-off layer of germinal epithelium. Outlines were traced on photographic prints which were subsequently bleached. Section numbers are as follows: no. 6508, 35-2-5; no. 6507, 11-1-4; no. 8101, 29-4-6; no. 406, 41-1-4; no. 4430, 24-4-6. ×300.

elsewhere in this same volume,[5, 6] and the reader is referred to those treatises. The germ cells are already present in horizon xv, where the present account starts. At that time they are found sparsely distributed among the proliferating cells of the coelomic epithelium. This is the youngest stage shown in figure 7, xviii. As the gonad progresses in development, the germ cells increase in number. But their apparent number depends on several factors, particularly on the thickness of the section, and it is not a favorable characteristic for grading the age of the embryo. The changes in the amount and arrangement of coelomic epithelium provide much more conspicuous characteristics of the developing gonad than the number and small cytological differences detectable in the primary germ cells themselves. It is the coelomic epithelium that provides the framework for the gonad, and it can be seen in figure 7, xviii that in the histogenesis of this framework the two-day changes between horizons, from xv to xviii, are sufficiently marked to make it possible to distinguish clearly between the gonads belonging to these successive age groups. In horizon xv the gonad is indistinctly demarcated from the underlying mesonephros. Over the gonadal area, however, there is a characteristic proliferation of the coelomic epithelium, producing an irregularity of the surface and at the same time giving off clusters of cells below which encroach on the underlying mesenchyme. The section illustrated is from an older member of the age group. A photograph of a section through the gonad of a younger member of horizon xvi, which merges closely with this, is shown on plate 1, xvi, along with illustrations of the embryo to which it belongs. Under this magnification one can distinguish the primary germ cells from the proliferating coelomic epithelium. With such a section there would be uncertainty as to whether to class the embryo as old xv or young xvi. The representative of horizon xvi, embryo no. 6507, in figure 7, xviii is more typical of the whole group. The ingrowth of coelomic epithelium is sufficiently advanced to produce a crescentic strip of gonadal tissue that is fairly well marked off from the mesonephric tissue. In horizon xvii, embryo no. 8101, the ingrowth of coelomic epithelium continues and the cells proliferating at the surface still project irregularly toward the coelomic cavity. The trabeculated epithelium has now the appearance of a dense framework in whose meshes are seen the primary germ cells. The dimensions of the gonad vary, of course, according to the region and direction of section. In general these dimensions are two or three times greater than those found in age group xvi, and the gonad is beginning now to acquire an oval form. On reaching horizon xviii the gonad has become an elongated oval, distinctly set off from the tissues of the mesonephros. The sections shown in figure 7, xviii are all enlarged to the same scale and thus the rate of growth between horizons can be seen by direct comparison. Among the older members of age group xviii one begins to encounter two types of gonadal framework, each of which is illustrated in the figure. This appears to be a sex difference, which now for the first time enters the developmental picture. Even among the older members of the age group this distinction cannot in all cases be made out. The two specimens selected for illustration are the best examples. It will be noticed that with recession in the proliferative activity of the coelomic epithelium, there is now a smooth surface layer of cells rather discretely set off from the reticulated framework of the gonad. It will also be seen that the cells of the framework are acquiring specialized characters, some of them becoming angioblasts. One cannot, however, rule out the possibility that the vasoformative cells may be invaders from the angioblastic system of the mesonephros. Although in this brief review of the histogenesis of the gonad we are leaving many interesting questions unanswered, enough has been seen to establish the point that as one advances from horizon to horizon a definitive state of gonadal development is consistently encountered for each age group.

### *Central Nervous System*

Sections through the caudal part of the brain have now acquired much of the essential form belonging to the mature structure, and there is no difficulty in recognizing the main regions. This level of development provides a good midway station to the familiar

[5] E. Witschi, Migration of the germ cells of human embryos from the yolk sac to the primitive gonadal folds, Carnegie Inst. Wash. Pub. 575, Contrib. to Embryol., vol. 32, pp. 67–80, 1948.

[6] J. Gillman, The development of the gonads in man, with a consideration of the role of fetal endocrines and the histogenesis of ovarian tumors, Carnegie Inst. Wash. Pub. 575, Contrib. to Embryol., vol. 32, pp. 81–131, 1948.

adult medulla oblongata. Particularly the trigeminal and other cranial nerves of the hindbrain exhibit in their emergence from the wall and in their central nuclear connections close similarity to the adult structure. Only the cerebellum and pons are still in a rudimentary state. Going farther rostral, as can be seen in figure 8, xviii, one can now distinguish a precocity and are actively growing and specializing. The marginal ridge (on the median wall) separating the relatively inactive thalamus from the more precocious subthalamus region is a conspicuous landmark at this time. From its position it may prove to have some relation to the well known thalamomammillary and tegmentomammillary tracts. If this point is to be

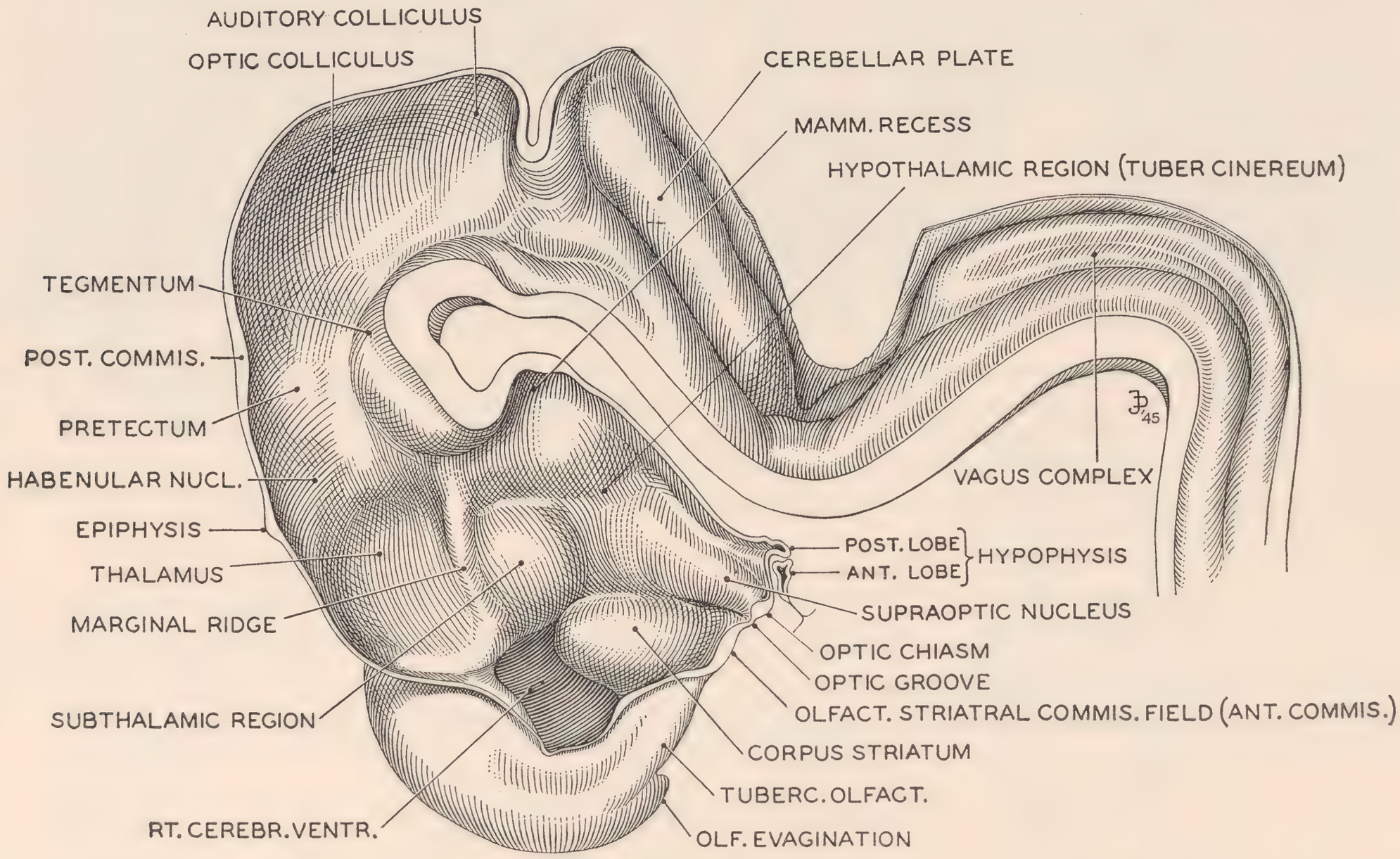



FIG. 8, xviii. Reconstruction of right half of brain of embryo (no. 492) belonging among older members of age group xviii. The basal plate continuing from the spinal cord and extending into the tegmentum has become thicker, and in transverse section the hypoglossal region begins to resemble sections of the mature hindbrain pattern. In the prominent midbrain region one can now recognize the primordium of the auditory colliculus as distinct from that of the optic colliculus. These dorsal parts, as well as the pretectum, habenular nucleus, and thalamus, are still backward as compared with the basal structures that are situated below and rostral to the marginal ridge. The latter has become more prominent than in the preceding age group. Advance in the hypophysis includes an elongated and folded neural lobe, and a characteristic expansion of the anterior lobe, whose slender stalk is still connected with the epithelium of the oral roof. The striated commissural field and optic chiasm retain their relative positions but as yet contain few, if any, commissural fibers. The olfactory evagination has become prominent. Drawing made by J. F. Didusch. Reconstruction made by O. O. Heard. ×12.5.

separate recess being derived from the caudal part of the optic colliculus and constituting the primordium of the auditory colliculus. Otherwise, rostral to the midbrain region, the changes from horizon xvii consist mostly in a further increase of about one-fourth in size and an increase in definition of the different parts from one another. The dorsal elements, the pretectum, the habenular region, the thalamus, and the neopallium are still thin and show scant differentiation, whereas the more basal masses show greater verified, the subsequent development of the marginal ridge must be followed into later stages.

In this horizon one can see the initiation of the anterior lobe of the epiphysis. This is illustrated in figure 9, xviii, showing sections of the epiphyseal region of two embryos (A and B) belonging to the median third of this age group. As one sees the neuroblasts migrate from the ependymal zone in the spinal cord to become the mantle layer, and as one sees the retinal cells migrate outward to become the "inner"

nuclear layer of the retina, so here also one sees cells migrating outward from the ependymal zone in the region of the epiphysis to form a segregated layer. A characteristic of this new layer is the tendency of its constituent cells to assume a follicular arrangement, explaining its subsequent glandular character. It is to be added that these minute cavities do not connect with the lumen of the epiphyseal evagination, but are the beginning of the characteristic tissue that is to form the anterior or rostral lobe of the epiphysis. In this proliferation and differentiation the anterior lobe is somewhat more precocious than the epiphyseal evagination itself. It is much later that the latter becomes elongated into a tubular ventricle.[7]

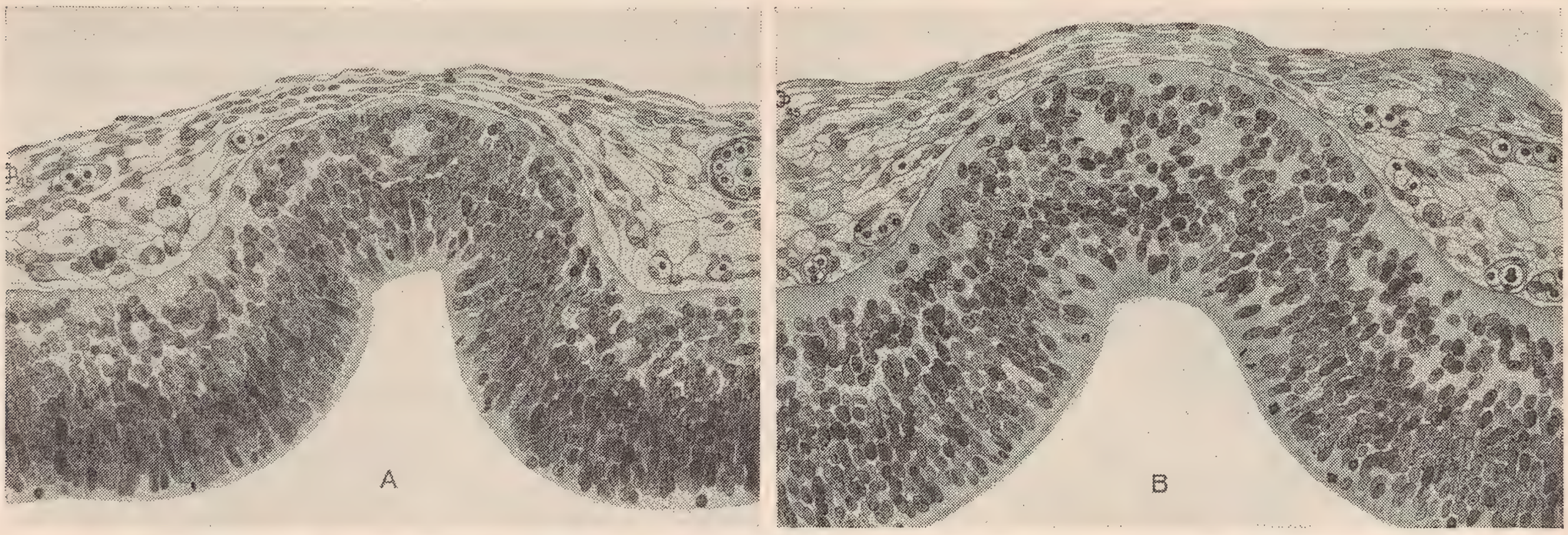



FIG. 9, xviii. Sections through the roof of the third ventricle of two embryos belonging in age group xviii, showing first steps in formation of the anterior lobe of the epiphysis. Nuclei migrate out from ependyma and form a mantle zone having a tendency to follicular arrangement of its cells. These minute follicles are not connected with the lumen of the evagination. The elongation of the epiphyseal evagination into a duct is relatively backward, and follows rather than precedes the formation of the anterior lobe. A, embryo no. 6527, section 11-3-4; B, embryo no. 7707, section 17-1-5. Drawings made by J. F. Didusch. ×200.

## SUMMARY OF HORIZON XVIII

As a summary of the developmental level of the embryonic organs in this horizon, there is shown in figure 10, xviii, in simple outline, a syndrome of characteristics which can be easily checked by microscopic examination of the sections of any embryo. If the embryo in question has these characters, then it belongs in this horizon. The heavily pigmented eye shows advanced migration forward of the inner nuclear layer of the retina. The secondary branches of the bronchi are bifurcated. The kidney has a long ureter and its pelvis exhibits branching of the calyces. Sections of the kidney show collecting ducts surrounded by sharply outlined condensed primordia in the process of forming tubules. Malpighian corpuscles are not present yet. The vomeronasal organ (Jacobson's) is represented in the form of a sharply evaginated groove. The membranous inner ear now has from one to three semicircular ducts.

Other prominent features of this period include the growth of the Müllerian duct and its rapid invasion down through the mesonephros, lateral to and in front of the mesonephric duct. In the younger half of the group it is 0.6 mm. or less in length, and in the older half its length ranges from 0.6 to 1.1 mm. In some of the more advanced members of the group one can recognize a sex difference in the gonads. In the heart, it is in this group that the aortic and pulmonary valves become sharply defined. Among the older members the membranous septum takes form and seals off the right from the left ventricle. In the central nervous system one can now recognize the primordium of the rostral lobe of the epiphysis. Also one can now speak of the posterior (auditory) colliculus. Finally, it is to be mentioned that in this group an irregular flange of epithelium grows inward from the nasomaxillary groove to form the pri-

[7] The reader is referred to the important paper by Hochstetter on the comparative embryology of the epiphysis, including man: F. Hochstetter, Die Entwicklung der Zirbeldrüse. Beiträge zur Entwicklungsgeschichte des menschlichen Gehirns, pt. 2, issue 1, Vienna and Leipzig, 1923.

mordium of the nasolacrimal duct, and at the same time the open nasal passage breaks through the buccnasal membrane and provides a free way from nostril through choana to pharynx. A respiratory bypass is thus established which is an instance of very liberal anticipation.

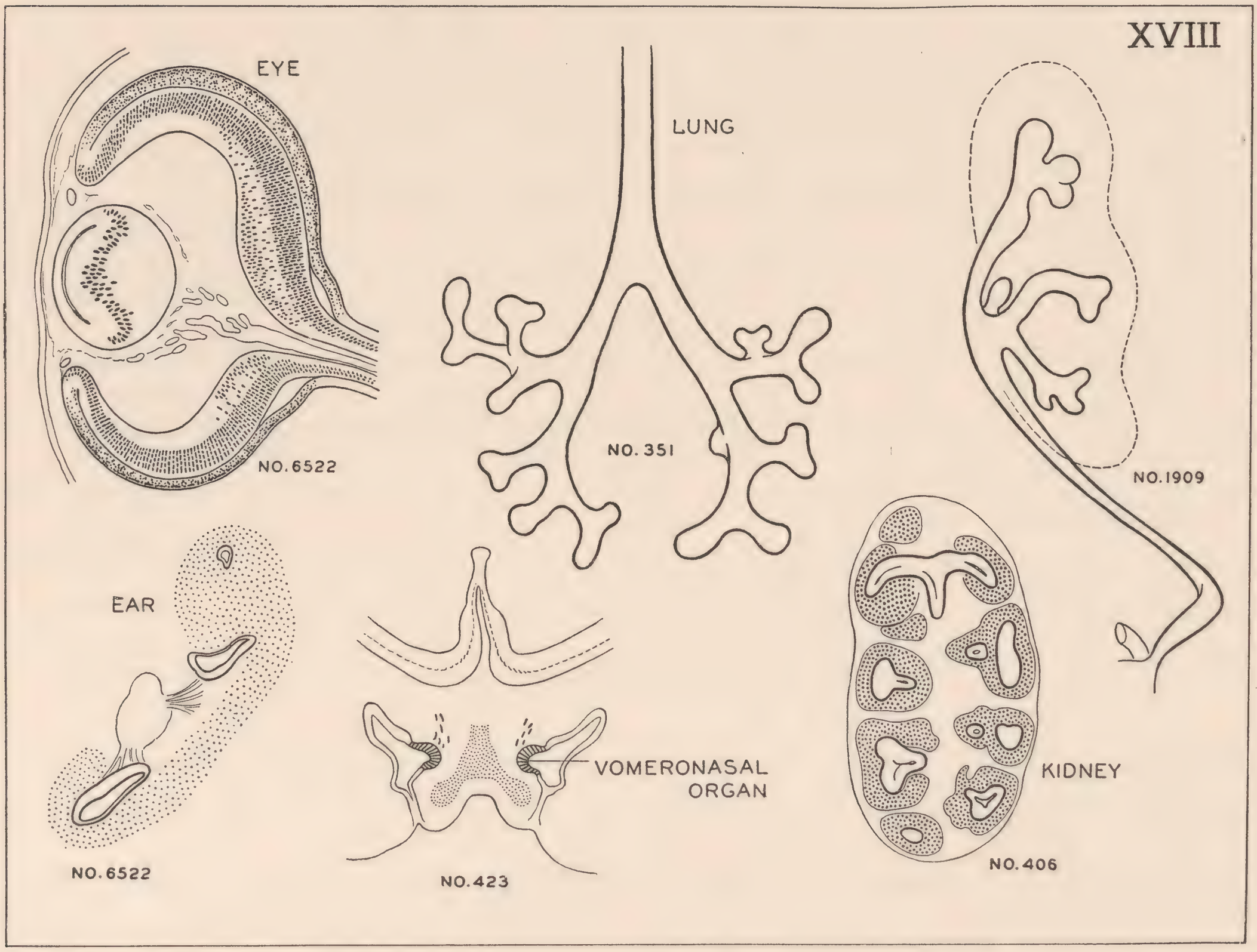



Fig. 10, xviii. Syndrome of structural features characteristic of age group xviii. In the eye the lens vesicle is reduced almost to a slit. The inner nuclear layer of the retina is widespread. The inner ear has one to three semicircular canals. There is a distinct vomeronasal organ (Jacobson's). In the lung there are beginning bronchi of the third order. In the kidney there are multiple calyces with sharply marked primordia of the convoluted tubules. As yet there are no definite glomeruli. The approximate magnifications are as follows: no. 6522, eye, ×66.6; no. 351, bronchial tree, ×66.6; no. 1909, frontal view left ureter, ×66.6; no. 6522, ear, ×34; no. 423, vomeronasal organ, ×17; no. 406, kidney, ×66.6. Compare with syndromes of preceding horizons.

## Normal Embryos in Carnegie Collection Belonging in Horizon XVIII

In addition to the data contained in the tables of embryos of earlier horizons, there is listed here also the number of semicircular ducts for each embryo and the length of the Müllerian ducts, these being convenient indices of relative level of development. As in previous lists, where an asterisk (*) follows the length of the embryo, this signifies that an estimated adjusted length is substituted for the length given in the laboratory records. The adjustment is made because of excessive shrinkage.

LIST OF EMBRYOS

| Serial no. | Size (mm.) | Grade | Fixation | Cutting medium | Plane | Thinness (microns) | Stain | No. semic. ducts | Length Mül. d. (mm.) | Date, donor, and remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| 109.. | E., 12.0*<br>Ov., 30 | Poor | Alc. | P | Trans. | 20 | Al. coch. | 1 | 0.4 | 1897, H. Cushing, Baltimore<br>Tubal; in youngest third |
| 144.. | E., 16.0*<br>Ov., 40 × 30 × 30 | Good | Formol | P | Sag. | 40 | Al. coch. | 3 | 0.85 | 1899, W. T. Watson, Baltimore<br>In oldest third |
| 175.. | E., 13.0<br>Ov., 30 × 25 × 25 | Poor | Alc. | P | Trans. | 20 | Al. coch. | 2 | 0.6 | 1900, H. J. Boldt, New York<br>Tubal; some maceration |
| 296.. | E., 17.0<br>Ov., ..... | Poor | Alc. | P | Frontal | 20 | Various | 3 | 0.85 | 1905, M. S. Haynes, Boston<br>Cytology poor; in oldest third |
| 317.. | E., 16.0<br>Ov., ..... | Good | Formol | P | Frontal | 20 | H.-or. G. | 2 | 0.7 | 1905, R. M. Pearce, Albany, N. Y.<br>In median group |
| 351.. | E., 14.0*<br>Ov., ..... | Good | Formol | P | Frontal | 250 | Slightly carmine | 2 | 0.38 | 1904, M. Brödel, Baltimore<br>Injected Berlin blue |
| 406.. | E., 16.0<br>Ov., 40 × 40 × 40 | Good | Formol | P | Sag. | 20 | H.-E. | 3 | 0.7 | 1907, J. L. Crawford, Cedar Rapids, Iowa<br>In oldest third; operative specimen |
| 423.. | E., 15.2<br>Ov., ..... | Good | Formol Zenker | P | Trans. | 50 | Carmine | 3 | 0.85 | 1904, J. H. Groshans, Baltimore |
| 424.. | E., 17.2<br>Ov., ..... | Good | Formol | P | Trans. | 50 | Carmine | 3 | 1.0 | 1904, M. Brödel, Baltimore<br>Double injection: older border line |
| 492.. | E., 16.8<br>Ov., 40 × 40 | Exc. | Zenker | P | Frontal | 40 | Al. coch. | 3 | 0.7 | 1911, F. V. Beitler, Halethorpe, Md.<br>Excellent India ink injection |
| 511.. | E., 16.0*<br>Ov., 38 × 32 × 32 | Good | Alc. | P | Sag. | 40 | Al. coch. | 3 | 1.1 | 1911, H. G. Steele, Keystone, W. Va.<br>Oldest in group; head injured |
| 670.. | E., 12.5<br>Ov., ..... | Poor | Alc. | P | Sag. | 50 | H.-E. | 3 | 1.0 | 1913, A. C. Smink, Baltimore<br>Tubal; near older border line |
| 719 | E., 15.0<br>Ov., 50 × 30 × 30 | Good | Formol | P | Trans. | 40 | Al. coch. | 2 | 0.6 | 1913, T. C. Smith, Washington, D. C.<br>Median position in group |
| 733.. | E., 15.0<br>Ov., 45 × 40 × 25 | Poor | Formol | P | Sag. | 50 | Al. coch. | 2 | 0.6 | 1913, W. S. Lemon, La Grange, Ill.<br>Median position in group |
| 841.. | E., 15.0<br>Ov., 18 × 16 × 9 | Good | Formol | P | Frontal<br>Trans. | 10<br>20 | H.-E.<br>carmine | 2 | 0.32 | 1914, W. C. McKee, Los Angeles, Calif.<br>Operative; head cut separately |
| 899.. | E., 16.0*<br>Ov., 30 × 18 × 15 | Good | Bouin | P | Sag. | 50 | Al. coch. | 3 | 0.65 | 1914, G. P. Evans, Baltimore<br>Tubal; head injured |
| 991.. | E., 17.0<br>Ov., ..... | Good | Formol | P | Sag. | 50 | H.<br>V. Gieson | 3 | 0.9 | 1914, R. L. Hoyt, Oxford, Md.<br>Near older border line |

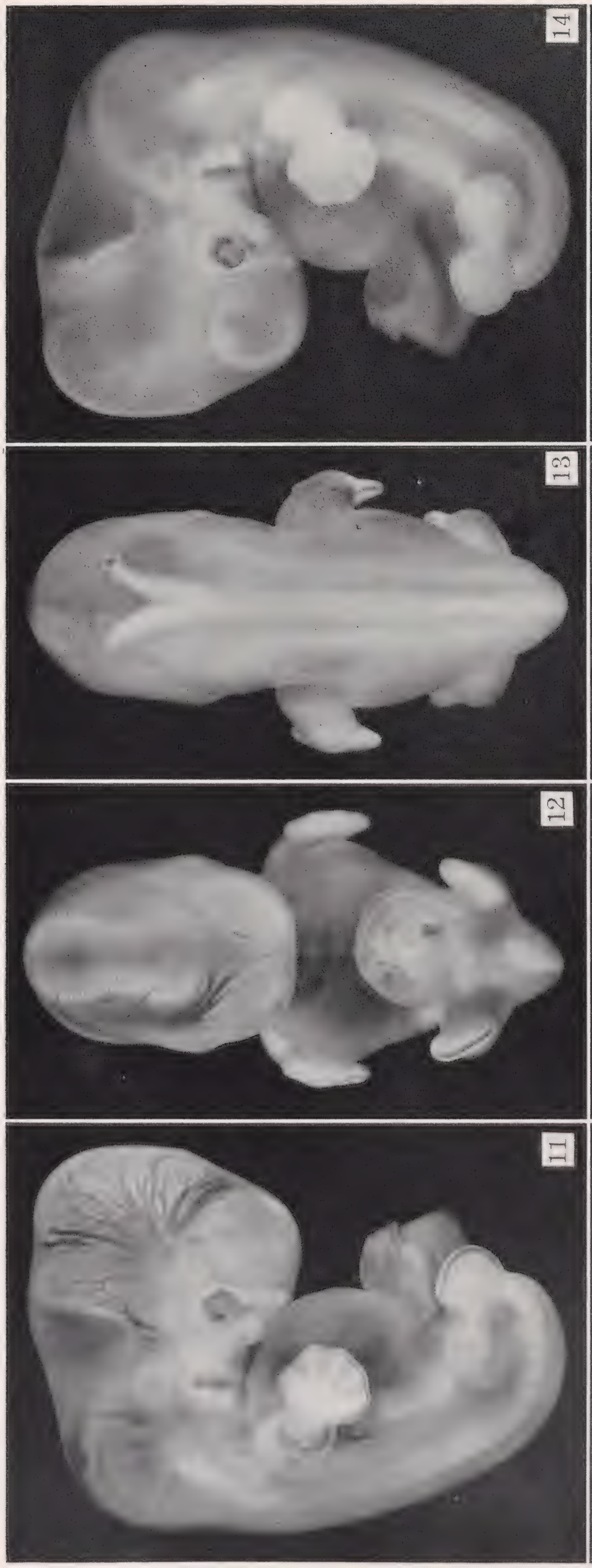
(*Continued on following page*)

LIST OF EMBRYOS—*Continued*

| Serial no. | Size (mm.) | Grade | Fixation | Cutting medium | Plane | Thinness (microns) | Stain | No. semic. ducts | Length Mül. d. (mm.) | Date, donor, and remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| 1909.. | E., 14.6<br>Ov., ..... | Good | Formol | P | Frontal | 20 | Al. coch. or. G. | 1 | 0.3 | 1917, R. W. Hammack, Los Angeles, Calif.<br>Near younger border line |
| 2673.. | E., 15.5<br>Ov., ..... | Good | Formol | P | Trans. | 40 | Al. coch. | 2 | 0.52 | 1919, J. H. Robinson, Baltimore<br>Median position in group |
| 4430.. | E., 14.0<br>Ov., 51 × 40 × 21 | Exc. | Corros. acetic | P | Trans. | 15 | Al. coch. or. G. | 3 | 0.9 | 1923, J. W. Williams, Baltimore<br>Among oldest third |
| 5542B | E., 16.0<br>Ov., 37 × 32 × 25 | Good | Formol | P | Trans. | 40 | Al. coch. | 2 | 0.7 | 1927, G. C. McCormick, Sparrows Point, Md.<br>One of twins; other twin abnormal |
| 5747.. | E., 15.2<br>Ov., 32 × 27 × 25 | Poor | Alc. formol | P | Sag. | 25 | Al. coch. | 2 | 0.25 | 1928, E. E. Williams, Possum Bend Center, Ky.<br>Border line of youngest and middle third |
| 5935A | E., 13.5<br>Ov., 40 × 30 × 30 | Good | Formol | P | Frontal | 40 | Al. coch. | 1 | 0.38 | 1929, J. W. Williams, Baltimore<br>One of twins; other twin stunted |
| 6522.. | E., 13.2*<br>Ov., ..... | Good | Corros. acetic | C-P | Frontal | 10 | Al. coch. | 3 | 0.8 | Border line median and oldest thirds; some tissues torn; others excellent |
| 6524.. | E., 11.7*<br>Ov., ..... | Exc. | Corros. acetic | C-P | Trans. | 10 | Al. coch. | 1 | 0.4 | In youngest third; some fragmentation, otherwise excellent |
| 6525.. | E., 13.8*<br>Ov., ..... | Exc. | Corros. acetic | C-P | Sag. | 8 | Al. coch. | 2 | 0.42 | Cut with difficulty; stain reaction weak, otherwise excellent |
| 6527.. | E., 14.4*<br>Ov., ..... | Exc. | Corros. acetic | C-P | Trans. | 15 | Al. coch. | 2 | 0.67 | Some mechanical damage; accessory nipple, left side |
| 6528.. | E., 13.4*<br>Ov., ..... | Exc. | Corros. acetic | C-P | Frontal | 8 | Al. coch. | 1 | 0.33 | Belongs in youngest third; some wrinkling of sections |
| 6529.. | E., 15.6*<br>Ov., ..... | Good | Corros. acetic | C-P | Frontal | 10 | Al. coch. | 2 | 0.4 | Beginning of median third; cytology of many structures excellent |
| 6533.. | E., 12.5*<br>Ov., ..... | Good | Corros. acetic | C-P | Sag. | 6, 8 10 | Al. coch. | 2 | 0.45 | Belongs in middle third |
| 6551.. | E., 18.0<br>Ov., ..... | Poor | Formol | P | Frontal | 40 | H.-E. | 3 | 0.8 | 1932, A. Guttmacher, Baltimore<br>Tubal |
| 7707.. | E., 14.5<br>Ov., 37 × 32 | Exc. | Bouin | C-P | Trans. | 10 | H.-E. phloxine | 2 | 0.54 | 1939, R. TeLinde, Baltimore<br>Operative; in median third |
| 8097.. | E., 15.5<br>Ov., 37 × 25 × 21 | Good | Formol | C-P | Trans. | 10 | H.-E. | 1 | 0.19 | 1942, A. Plaut, New York<br>Youngest in the horizon |
| 8172.. | E., 16.5<br>Ov., ..... | Exc. | Bouin | C-P | Trans. | 20 | H.-E. | 3 | 0.58 | 1943, E. H. Richardson, Baltimore<br>Operative; border line; old |
| 8235.. | E., 14.0*<br>Ov., ..... | Good | Bouin | C-P | Sag. | 10 | H.-E. Mallory | 2 | 0.25 | 1944, C. H. Peckham, Coopers-town, N. Y.<br>Tubal; second canal very young |

FIGS. 11–14. Four views of a younger member (embryo no. 8097) of age group xviii. As it is somewhat transparent, one can see the blood-vessel patterns and details of inner structure. The broad triangular inner ear can be seen in the profile views, also the trigeminal nerve and details of the eye. One cannot speak of a primordial skull except in the ventral part of the head. Since they are so thinly covered, one can see many details in the form of the brain parts, notably the cerebral hemispheres. In figure 12, above, one sees the cavity of the collicular region and isthmus leading to the hindbrain. Rostral, below in the picture, is the dark mammillary recess with the infundibular floor continuing forward. ×5.

FIGS. 15–18. Four views of embryo no. 7707, an older member of age group xviii. Owing to the character of the fixation, the tissues are opaque, making the specimen favorable for study of surface modeling. In preceding age groups we have seen the embryo becoming wider from side to side, and, as seen in figures 16 and 17, it is now more or less cuboidal. The digital rays have become more distinct and the rim of the hand plate is moderately crenated; also one can begin to recognize the elbow bend. Toe rays are seen in figure 18. The small condensation masses creeping forward over the retina give a typical appearance to the eye, and one must admit a beginning of the upper eyelid. The three-hillock plate, to the rear of the auditory groove, exhibits different stages in its resolution into definitive parts of the external ear. Finally, the caudal end of the spine is now separating into the permanent thicker part and a slender terminal filament that will disappear. ×5.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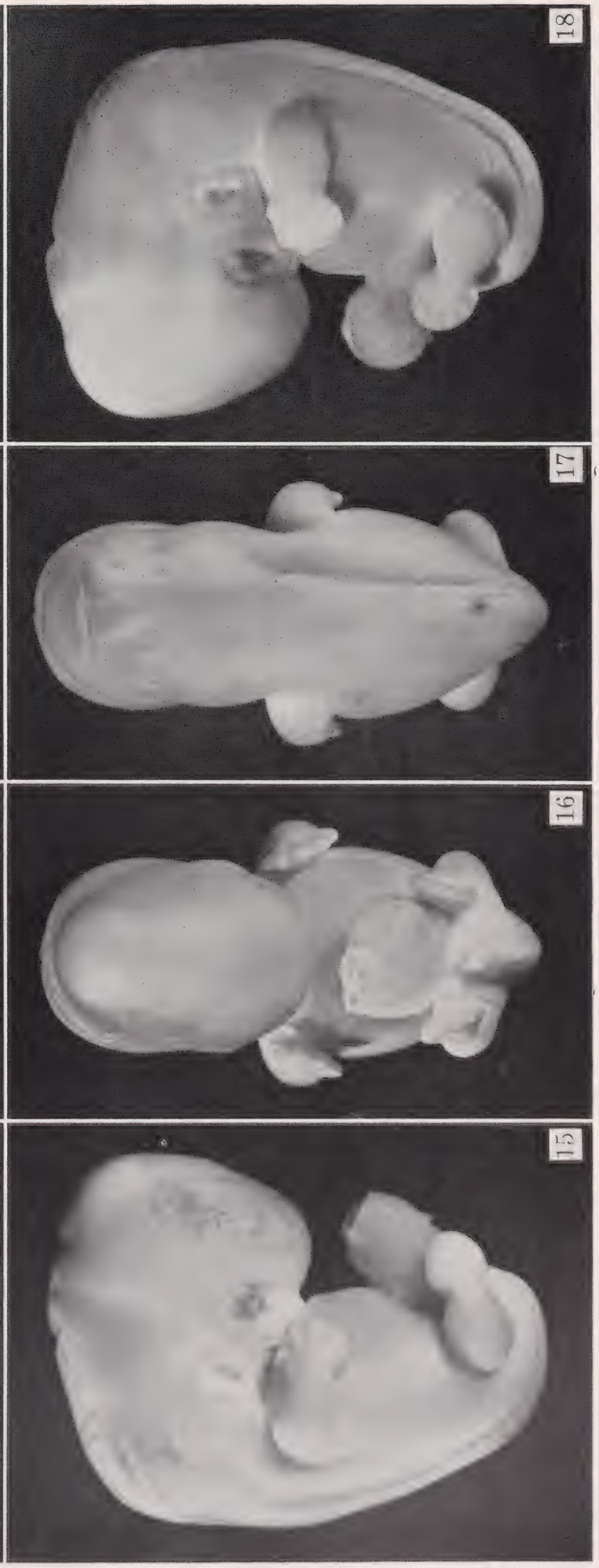
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Photographs illustrating the variations in form met with in age group xviii. In studying these specimens one must keep in mind those appearances that are the result of fixation, shrinkage, and the original condition of the embryo. All these specimens have been sectioned, and in their microscopic structure qualify as members of this horizon, the horizon in which semicircular ducts are acquired by the inner ear, the horizon in which the Müllerian duct descends along the margin of the mesonephros, and in which the first signs of sex difference can be detected in the gonads. In general the embryos in the upper part of the plate are younger and those toward the bottom are older. The embryo shown in figure 27 is near the border line. It was fixed in a corrosive acetic mixture, which accentuates its surface features. One can see the right nipple just under the arm.

The embryo numbers and enlargements are as follows:

FIG. 19. No. 1909, ×4.
FIG. 20. No. 5935A, ×5.
FIG. 21. No. 6525, ×5.
FIG. 22. No. 6528, ×5.
FIG. 23. No. 5542B, ×4.
FIG. 24. No. 6533, ×5.
FIG. 25. No. 6522, ×5.
FIG. 26. No. 6529, ×4.
FIG. 27. No. 4430, ×5.

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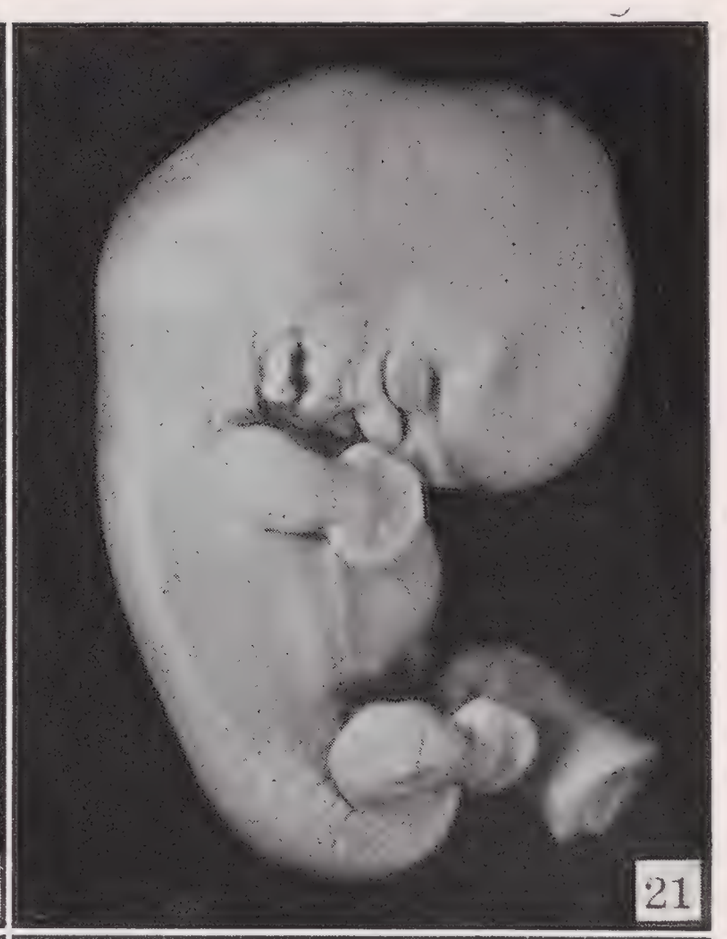
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27

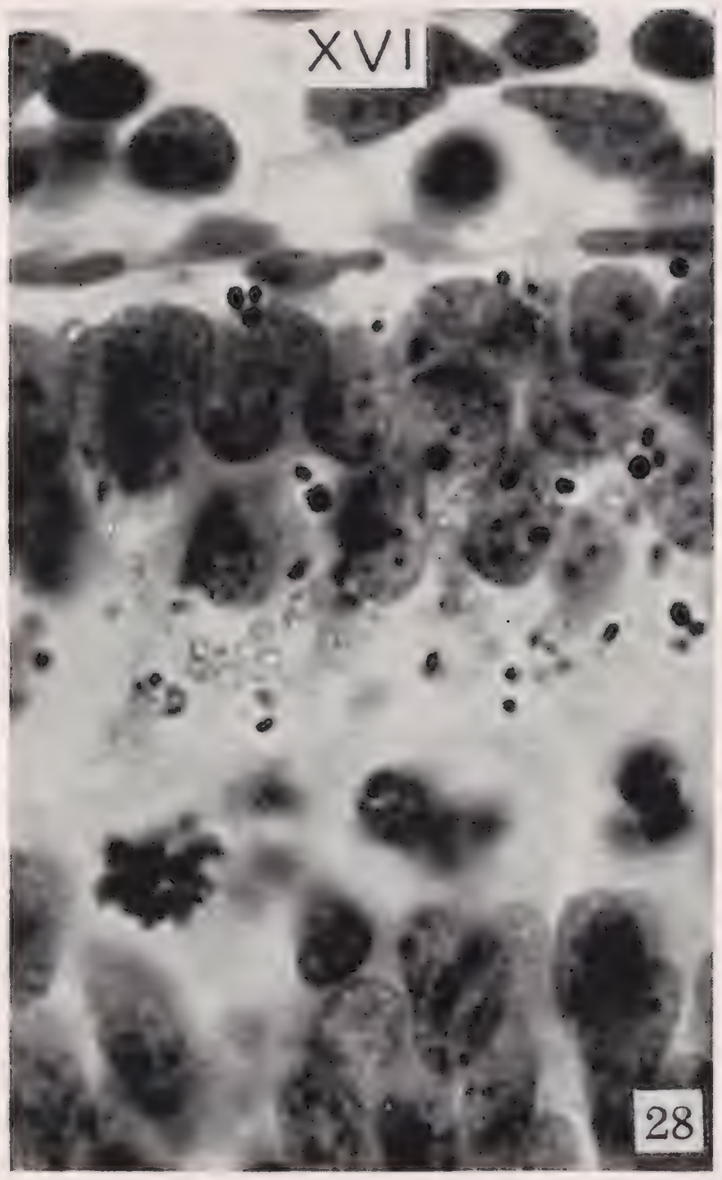
The three sections at the top of the plate show the deposit of pigment granules in the outer layer of the retina, as seen under a magnification of 1500, in three successive age groups. The first, figure 28, has but a few distinct granules, and some refractive colorless droplets. This is the appearance of horizon xvi, when pigment is first seen. In horizon xvii (fig. 29) there are more granules, and they show a tendency to cluster at the outer surface. In horizon xviii (fig. 30) one commonly finds a crust of granules on the outer surface. In judging the amount of pigment, allowance must be made for section thickness. Angioblasts can be seen on the outer surface of the pigmented layer of the retina. At the bottom of the photographs is seen in each instance the neural layer of the retina. Embryo and section numbers are as follows:

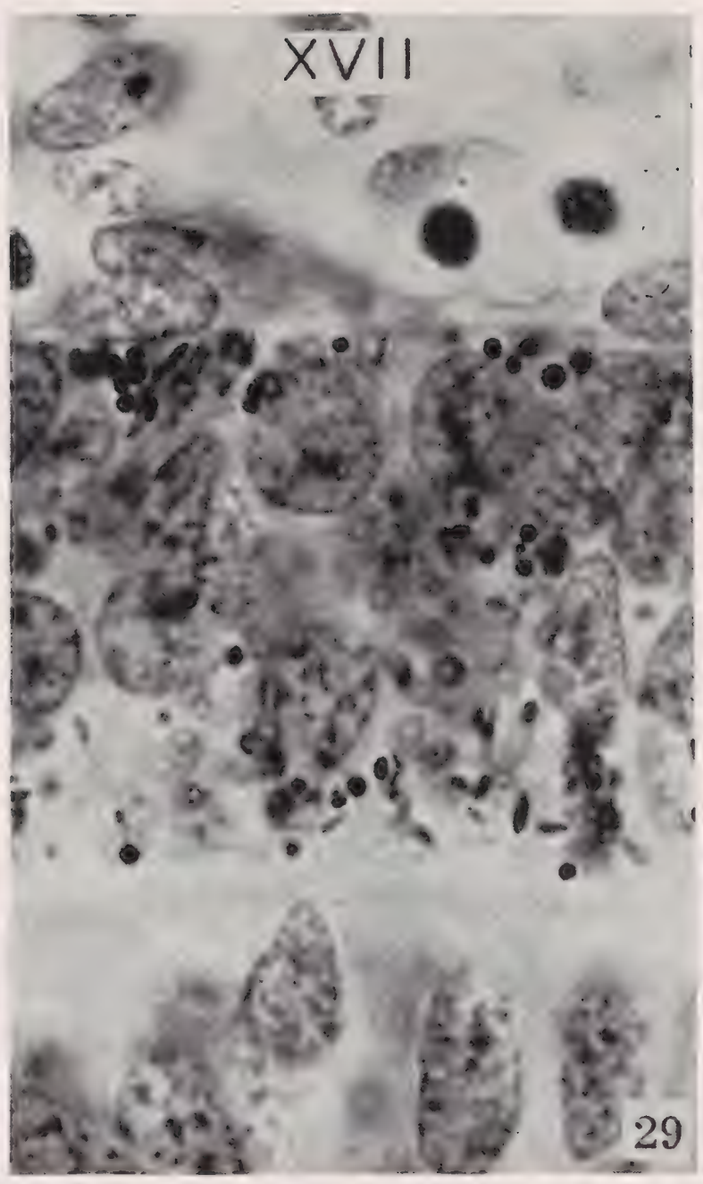
FIG. 28. No. 6507, 7-1-3, 10 microns.

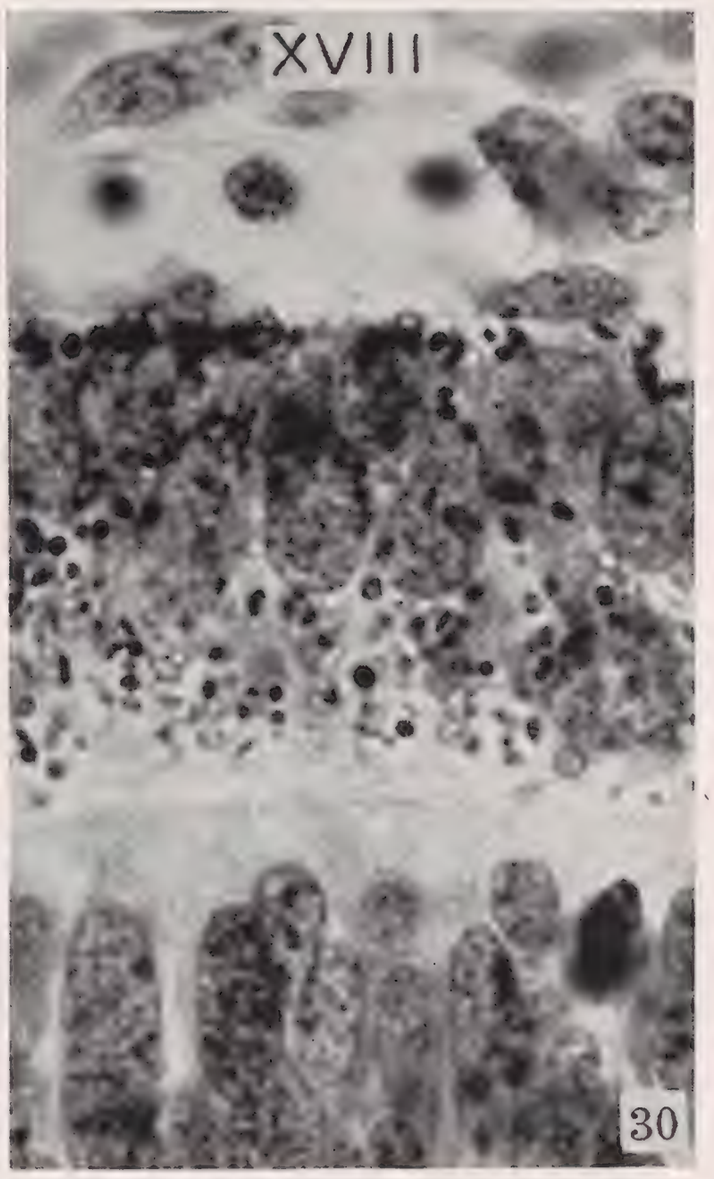
FIG. 29. No. 6520, 23-3-5, 10 microns.

FIG. 30. No. 6528, 25-2-3, 8 microns.

FIG. 31. Section through an eye typical of age group xviii. The forward migration of retinal cells to become the inner nuclear layer is now widespread. The cavity of the lens vesicle is reduced to a slit, with a corresponding building up of the disk of lens fibers. One can see the characteristic distribution of lenticular nuclei and the fiber lines. It is to be noted that the mesenchyme has begun to invade the interval between lens epithelium and surface epithelium, a prelude to cornea formation. Embryo no. 6527, section 19-3-5, ×200.


XVI
28


XVII
29


XVIII
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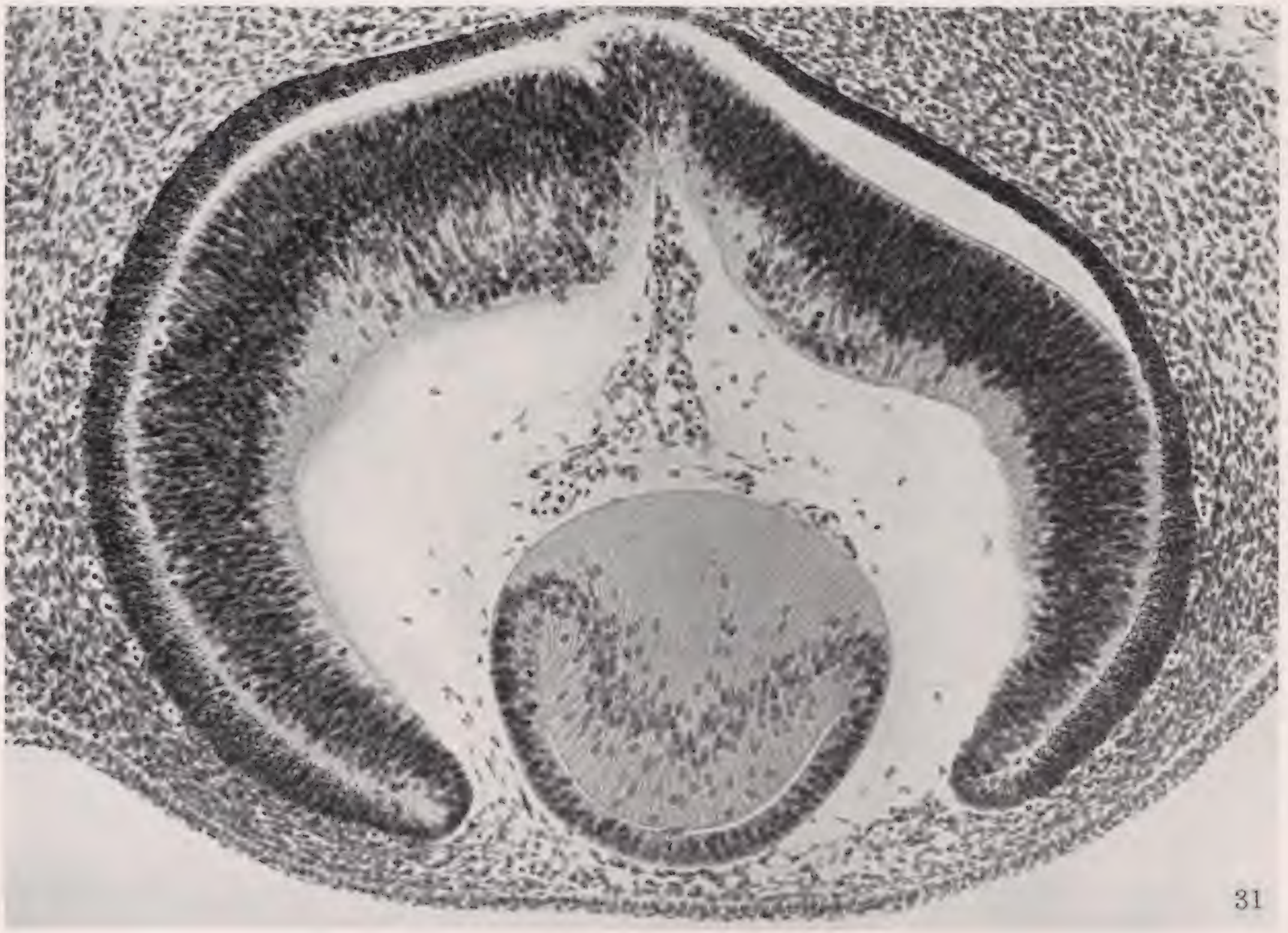
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CONTRIBUTIONS TO EMBRYOLOGY, NO. 212

# THE DEVELOPMENT OF THE CRANIAL ARTERIES IN THE HUMAN EMBRYO

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With five plates (two colored) and thirteen text figures

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# THE DEVELOPMENT OF THE CRANIAL ARTERIES IN THE HUMAN EMBRYO[1]

## INTRODUCTION

The literature is singularly incomplete respecting the development of the cerebral arteries. This became apparent in connection with a study of the circle of Willis and its anomalies prepared by the writer for a monograph on intracranial arterial aneurysms by Dandy (1944). The initial interest in the embryonic derivation of the arteries concerned led to an analysis of all the cranial arteries, which is the scope of this paper. Since Evans' account of the head arteries in the Keibel and Mall *Manual* (1912), based on his own investigations and those of Tandler (1902), Mall (1905), and others, there have appeared two well known papers. Streeter (1918) described the underlying principles in the formation of the cranial vascular system and the adjustment of the blood vessels to growth changes in the brain and environment. He was chiefly concerned, however, with the development of the drainage channels, in particular the dural sinuses. Congdon (1922) fully described the evolution of the aortic arch system, including significant features in the origin of three cerebral arteries, the carotid, the basilar, and the vertebral. To supplement these fundamental studies, seven stages in the embryonic configuration of all the arteries in the region of the head are presented. During the preparation of this outline, three individual arteries, two of them temporary and one permanent, required particularly intensive study. The ophthalmic artery in recognizable adult form emerges relatively late in the embryo and only after a series of modifications which involve the temporary stapedial artery. The formation of the human stapedial artery and certain details concerning its retrogression have not been previously published. Another temporary artery, here named the "primitive trigeminal," was observed to be often conspicuous and always significant in relation to the development of several permanent arteries and is described for the first time as found in the human embryo. A recapitulation of the essential features of these individual arteries, discussed fully in the last three sections of this paper, will be found in the opening outline of stages, which covers all the cranial arteries.

The present study is based on the graphic, profile reconstructions of 22 sectioned embryos of the Carnegie Collection. These range from 3½ to 7 weeks (estimated ovulation age) and from 3 mm. (20 somites) in crown-rump length, when the primitive internal carotid artery appears, to approximately 40 mm., when the definitive adult origin of essentially all the cranial arteries is established. Using the enlarged photographic prints of serial sections of the embryos (or, in a few cases, tracings obtained on the Edinger apparatus), the reconstructions were built up by successive tracings on transparent paper or were plotted on millimeter paper. Employing both methods, many embryos were reconstructed in two or three planes (table 1). Thus, the standard lateral, horizontal, and coronal views gave a working basis with the advantages of the three-dimensional model, and, in addition, permitted much detail and accuracy in reference to the smaller arteries and their environmental relations. Certain details were clarified by microscopic study of other embryos not available for reconstruction; in two of these cases, as noted in the text, a partial freehand reconstruction on millimeter paper was feasible. The work was occasionally facilitated by existing models of the brain, aortic arches, or cerebral vessels of several embryos of the series, but none of these were used in preparing the present illustrations, which were made by the writer.

I wish to thank all those to whom I am indebted in connection with the preparation of this paper, which would have been impossible without the many courtesies, critical judgment, or assistance of Dr. George W. Corner, Dr. George L. Streeter, and Mr. James F. Didusch, of the Carnegie Institution of Washington. Particular thanks are also extended to Dr. David Bodian, Dr. William L. Straus, Jr., and Dr. Joseph Earle Moore, for review of the manuscript and many helpful suggestions. It is a great pleasure to express my gratitude to the late Dr. Walter E. Dandy, with whom I was long associated as a medical illustrator,

[1] The author was formerly on the Neurological Service of Dr. Walter E. Dandy, Johns Hopkins Hospital and Medical School. The embryological material was provided by the Department of Embryology of the Carnegie Institution of Washington.

and who inspired the study and generously made the grant necessary for its completion. Finally, I wish to pay tribute to the memory of the late Professor Max Brödel, whose personal influence I had the privilege of enjoying much longer than was the good fortune of most of his students.

## STAGES IN THE DEVELOPMENT OF THE CRANIAL ARTERIES

The order of appearance of all the definitive cranial arteries cannot be stated categorically because of the well known existence of individual variation, particularly in the younger embryos. It was found, however, that the sequence of events fell logically into eight stages including the adult configuration, and more than one embryo, including several from the literature, was considered in reference to each. Table 1

### TABLE 1

EMBRYOS GRAPHICALLY RECONSTRUCTED. CORRELATION OF SIZE AND AGE GROUP WITH THE DEVELOPMENT OF THE AORTIC ARCH SYSTEM AND THE CRANIAL ARTERIES

| EMBRYO NO. | LENGTH (MM.) | VIEWS RECONSTRUCTED | FIGURE | CHARACTERISTIC FEATURES OF: | |
|---|---|---|---|---|---|
| | | | | Individual embryo | Stage in general |
| TIME OF ESTABLISHMENT OF 1ST ARCH; 2D ARCH BEGINNING; ESTIMATED OVULATION AGE* 24 ± 1 DAYS | | | | | |
| 2053.. (xi†) | 3 (20 som.) | Bilat. Coron. | 37, pl. 4 43, pl. 5 | Primitive internal carotid and trigeminal arteries are branches of first arch; latter artery supplies cranial end of primordial hindbrain channel. | Establishment of primordial vascular system. Primordial hindbrain channel serves temporarily as vein for head; it is supplied by presegmental branches of paired ("dorsal") aorta. |
| TIME OF ESTABLISHMENT OF 2D AND 3D ARCHES; BEGINNING INVOLUTION OF 1ST ARCH; ESTIMATED OVULATION AGE 28 ± 1 DAYS | | | | | |
| 836.. (xiii) | 4 | Bilat. Horiz. | 38, pl. 4 39, pl. 4 | Longitudinal neural arteries in formation, supplied by trigeminal and otic arteries (presegmental branches of paired "dorsal" aorta). | First stage in differentiation of supply and drainage channels. Disappearance of primordial hindbrain channel; resulting emergence of bilateral longitudinal neural arteries, which precede the basilar. |
| TIME OF ESTABLISHMENT OF 4TH ARCH; PULMONARY ARCH IN FORMATION; INVOLUTION OF 1ST AND 2D ARCHES; ESTIMATED OVULATION AGE 29 ± 1 DAYS | | | | | |
| 588.. (xiii) | 4 | Bilat. Horiz. Coron. | 1*a* 1*b* 44, pl. 5 | Early form of mandibular and hyoid arteries; dorsal and ventral ends more or less continuous. | Bilateral longitudinal neural artery supplied by trigeminal artery cranially, reinforced by first cervical segmental artery caudally. Establishment of cranial and caudal divisions of internal carotid artery. (STAGE 1: fig. 14, pl. 1; fig. 22, pl. 2.) |
| 1380.. (xiv) | 5.7 | Bilat. Coron. | ..... ..... | Later form of mandibular and hyoid arteries; dorsal remnants only. Ventral pharyngeal artery. | |
| 2841.. (xiv) | 5.3 | L. lat. Horiz. | 2*a* 2*b*; 30, pl. 3 | Dorsal remnants of mandibular (small) and hyoid (large) arteries. Ventral pharyngeal artery. | Basilar artery in formation from bilateral neural arteries, which are supplied cranially by new posterior communicating arteries (caudal divisions of carotids), sometimes reinforced by dwindling trigeminal and hypoglossal arteries. (STAGE 2: fig. 15, pl. 1; fig. 23, pl. 2.) |
| 3960.. (xiv) | 5.5 | R. lat. Horiz. | 3*a* 3*b*, *c* | Large ventral pharyngeal artery supplies major substance of first two pharyngeal bars. Primitive dorsal ophthalmic artery to lens primordium. | |

* Estimates based on Heuser and Streeter's (1941) curve of length and age in the macaque.

† Roman numerals indicate developmental horizons of the Carnegie Collection, Streeter (1942, 1945, 1948). Groups xix through xxi are provisional.

(*Continued on following page*)

TABLE 1—*Continued*

| Embryo no. | Length (mm.) | Views reconstructed | Figure | Characteristic features of: Individual embryo | Characteristic features of: Stage in general |
|---|---|---|---|---|---|
| **Time of establishment of pulmonary arch; splitting of aortic sac; estimated ovulation age 32 ± 1 days** | | | | | |
| 4782.. (xv) | 9 | Horiz. | ..... | Intermediate stage in formation of basilar artery. Caroticobasilar (trigeminal) anastomosis large on one side. | Vertebral artery in formation. Beginning differentiation of cerebral arteries. Cranial division of internal carotid constitutes a primitive olfactory artery. Optic supply still plexiform but includes two primitive ophthalmic branches of carotid, one dorsal and one ventral. Stem of hyoid artery now at cranial edge of hyoid bar. Ventral pharyngeal artery accompanies mandibular end of chorda tympani nerve to mandibular root of fifth nerve. (Stage 3: fig. 16, pl. 1; fig. 24, pl. 2.) |
| 617.. (xvi) | 7 | R. lat.<br>Horiz. | .....<br>4*b*; 31, pl. 3 | Late stage in formation of basilar; irregular with many islands. | |
| 1121.. (xvi) | 11.8 | Bilat.<br>Horiz.<br>Coron. | .....<br>.....<br>45, pl. 5 | Island at caudal end of basilar. Interrupted remnants of caroticobasilar anastomosis. Early stage in formation of vertebral artery. | |
| 163.. (xvi) | 9 | L. lat.<br>Horiz. | 4*a*<br>..... | Basilar completed. Basilar remnant of caroticobasilar anastomosis. Anastomosis of cervical segmentals to form vertebral artery nearly complete. | |
| **Time of interruption of right pulmonary arch and of "branchial period"; estimated ovulation age 35 ± 1 days** | | | | | |
| 1771.. (xvii) | 12.5 | Bilat.<br>Horiz. | 5*a*<br>5*b* | Late stage in formation of vertebral artery. Differentiation of cerebral arteries well advanced. | Vertebral artery complete but irregular; origin still low on paired aorta. A secondary branch of the hyoid artery constitutes the new stapedial artery, which has anastomosed with distal (mandibular) end of ventral pharyngeal artery at mandibular nerve root. Early stage in formation of anterior cerebral as a secondary branch of primitive olfactory artery. Derivative of primitive dorsal ophthalmic artery enters fetal ocular cleft as definitive hyaloid artery. Components of external carotid artery. (Stage 4: fig. 17, pl. 1; fig. 25, pl. 2.) |
| 544.. (xvii) | 11.5 | Bilat.<br>Horiz. | 40, pl. 4<br>41, pl. 4 | All cerebral arteries relatively primitive and plexiform. Bilateral retention of caroticobasilar (trigeminal) anastomosis in prominent form in both embryos. | |
| 940.. (xvii) | 14 | Bilat.<br>Horiz.<br>Coron. | 6<br>32, pl. 3; 42, pl. 4<br>46, pl. 5 | | |
| 6520.. (xvii) | 12.9 | Horiz. | ..... | Differentiation of cerebral arteries advanced except at caudal part of hindbrain. | |
| **End of period of rapid descent of heart and arteries; aortic arch sharply bent; estimated ovulation age 40 ± 1 days** | | | | | |
| 144.. (xviii) | 16 | L. lat. | ..... | These embryos are similar and resemble the 15.5-mm. and 17.8-mm. embryos of Evans (1912) and Thyng (1914), respectively. | Vertebral artery regular with origin moved to level of ductus arteriosus. Stapedial artery has three (mandibular, maxillary, and ophthalmic) branches. New permanent stem of ophthalmic artery, including primary optic branches. Middle and anterior cerebrals advanced. Primitive olfactory artery dwindling. Plexus in region of anterior communicating artery. Most cerebral arteries recognizable. Four or five branches of external carotid. (Stage 5: fig. 18, pl. 1; fig. 26, pl. 2.) |
| 1390.. (xix) | 18 | Bilat.<br>Horiz. | 7*a*<br>7*b*; 33, pl. 3 | | |

(*Continued on following page*)

TABLE 1—*Continued*

| Embryo no. | Length (mm.) | Views reconstructed | Figure | Characteristic features of: Individual embryo | Stage in general |
|---|---|---|---|---|---|
| **Summit of definitive aortic arch at superior thoracic aperture; estimated ovulation age 44 ± 1 days** | | | | | |
| 6202.. (xx) | 21 | Bilat. Horiz. | ..... 34, pl. 3 | Anterior communicating artery plexiform. | Circle of Willis completed by anterior communicating artery. Anterior cerebrals extend up between cerebral hemispheres; medial striate branches are remnants of primitive olfactory artery. Formation of orbital branches of ophthalmic artery by anastomosis between ophthalmic stem and supraorbital stapedial division. Stem of middle meningeal artery formed by anastomosis between maxillomandibular division of stapedial and internal maxillary artery. Most external carotid branches definitive. (Stage 6: fig. 19, pl. 1; fig. 27, pl. 2.) |
| 460.. (xx) | 21 | Horiz. | ..... | Anterior communicating artery present as single large channel. Island in basilar at fifth nerve level. | |
| 966.. (xx) | 23 | L. lat. Coron. | ..... 9; 47, pl. 5 | Anterior communicating artery a single channel. Plexiform remnants of trigeminal anastomosis. | |
| 632.. (xxi) | 24 | Bilat. Horiz. | 8*a* 8*b;* 35, pl. 3 | Anterior communicating artery large but duplicated. Interruption of stem of stapedial artery. | |
| **Origin of right and left pulmonary branches in contact through most of their length; estimated ovulation age 52 ± 1 days** | | | | | |
| 6203.. | 39 | L. lat. Horiz. | 10*a* 10*b;* 36, pl. 3 | Configuration of ophthalmic artery adult on left side, but primitive on right. Definitive medial striate arteries (remnants of primitive olfactory artery). | Circle of Willis recognizable in ventral (base) view of brain. Ophthalmic artery complete including lacrimal branch (segment of supraorbital division of stapedial). All stapedial branches annexed by ophthalmic and external carotid arteries. Stem of origin of all cerebral arteries is adult, although destination of several, notably the posterior cerebral and both inferior cerebellar arteries, conforms to the immature cerebral and cerebellar hemispheres. (Stage 7: fig. 20, pl. 1; fig 28, pl. 2.) |
| 886.. | 43 | L. lat. Horiz. Coron. | 10*a* 10*b* 48, pl. 5 | Small basilar remnant of trigeminal anastomosis. Striate branches of anterior cerebral in formation from remnants of primitive olfactory artery. | |

shows the reconstructed embryos arranged in order of general development, and correlates successive stages of the cranial arteries and the aortic arch system. This table, patterned after that of Congdon (1922), includes 14 of the same embryos utilized by that author. Classification of the remaining 8 was determined from the development of the brain, arteries, and external form. The embryos listed in table 1, and noted in the text, are also classified in the age groups (age group xi, xii, etc.) currently being determined by Dr. Streeter (1942, 1945, this volume, and personal communication). These groups, of which 23 in all are contemplated, will carry through the first 7 weeks and will include embryos up to approximately 40 mm., "beyond [which] time the weekly increment in growth and weight is large enough to provide an adequate index of relative development."

## Stage 1, Embryos Approximately 4–5 mm., Age Groups XIII, XIV

An embryo of 4 mm. (no. 588, fig. 1), which Streeter described as illustrative of the primary type of cranial circulation, constitutes a logical stage with which to begin the present outline. The arterial system is somewhat more advanced than in no. 836, which is also in age group xiii and will be described below (pl. 4, figs. 38, 39); and is less advanced than in no. 1380, of 5.7 mm., in age group xiv. In no. 1380 the optic vesicle is at a later stage, but, although no vessels could be traced in the substance of its first two

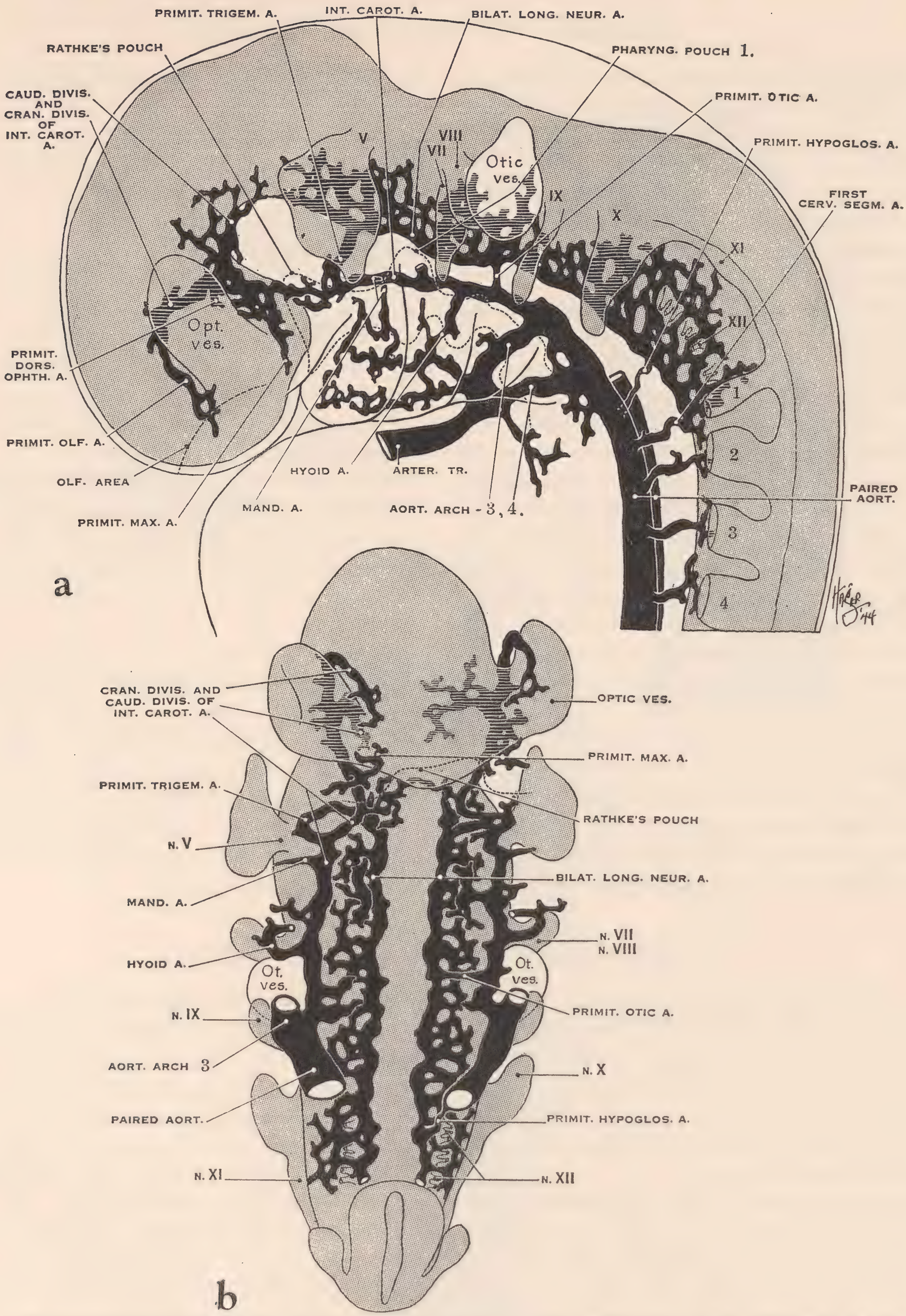


FIG. 1. Stage 1. Graphic reconstruction of the cranial arteries in a 4-mm. embryo (no. 588). Following the involution of the first two aortic arches, now represented by mandibular and hyoid arteries, the terminal end of the paired aorta of earlier stages (figs. 37, 38, pl. 4) is recognizable as the internal carotid artery originating from the third arch (*a*). The forebrain and optic vesicle are supplied by the primitive maxillary branch and the primary cranial division of the carotid; the caudal division courses over the midbrain. The emerging bilateral neural artery on the hindbrain wall (*b*) is also supplied by the carotid through the primitive trigeminal branch, supplemented by other presegmental branches of the original paired aorta in the otic and hypoglossal regions. The caudal end of the neural artery is fed by the first cervical segmental artery. ×50.

pharyngeal bars, this embryo and no. 588 are otherwise similar in arterial and nervous development of the head, and the following description holds true in general for both embryos.

Since the first and second aortic arches are in early stages of involution, the portion of paired ("dorsal") aorta extending from the third arch now represents the primitive internal carotid artery. In addition to the mandibular and hyoid arteries (replacing the first two aortic arches), there are other conspicuous mesial and lateral offshoots, and all together the carotid has the appearance of having just emerged from a plexus. At the trigeminal ganglion, the carotid divides into two branches. One, the cerebral continuation of the carotid artery, extends craniomesially toward Rathke's pouch, around which it is connected with the opposite carotid by plexiform channels. The other is the primitive trigeminal artery, which extends in a generally dorsal direction to join the bilateral longitudinal neural artery on the hindbrain wall. Just beyond the base of Rathke's diverticulum, the carotid gives off the primitive maxillary artery, which courses ventrally along the forebrain wall to the base of the optic vesicle. Near the summit of the vesicle, the carotid forms the two divisions described by De Vriese (1905) as typical of arterial development in the vertebrates. The cranial division curves around in front of the optic vesicle to terminate at the olfactory area, and the caudal division ends in a plexus at the mesencephalon. A small offshoot near the junction of the carotid division passes to the mesial face of the optic vesicle and represents a primitive ophthalmic artery, but supply to the future eye is highly plexiform at this stage and is contributed by both the carotid and its primitive maxillary branch.

In the hindbrain region, the bilateral longitudinal neural arteries have just become recognizable bordering a ventral mid-line nonvascular strip (fig. 1*b*) and are everywhere connected laterally with the primitive hindbrain plexus. The neural arteries are supplied cranially by the trigeminal divisions of the carotid, noted above, and caudally by the first cervical segmental arteries. This supply to the neural arteries is reinforced to a variable extent by remnants of two highly transitory presegmental branches of the paired aorta, namely, the primitive otic artery, at the level of the acoustic nerve and otocyst, and the primitive hypoglossal artery, in the region of the twelfth nerve.

To summarize, this stage (fig. 1) shows the early configuration of the internal carotid arteries coincident with the initial involution of the first and second aortic arches, and the early channels of supply to the bilateral longitudinal neural arteries which precede the development of the basilar artery. It may be compared with Streeter's (1918) illustrations (his pl. 2) of the venous channels modeled from the same embryo.

### Stage 2, Embryos Approximately 5–6 mm., Age Group XIV

An embryo of 5.3 mm. (no. 2841, fig. 2) illustrates an early stage in the formation of the basilar artery as described by Congdon (1922). Another embryo of 5.5 mm. in the same age group (no. 3960, fig. 3), with an unusually complete natural injection of the vessels, proved to be a valuable supplement in reference to several important arteries.

The internal carotid is now well defined and extends from the third aortic arch. Its mandibular branch is reduced to a small remnant, but the hyoid branch, which later becomes the carotid stem of the stapedial artery, is conspicuous. There is a prominent vessel in the general position of the ventral part of the first two aortic arches. This is the "ventral pharyngeal artery" (Congdon, 1922), which extends craniolaterally from the aortic sac at the mid-line to the mandibular root of the fifth nerve, and, as will be shown later, has an important role in the formation of the stapedial and external carotid arteries.

In one embryo (no. 3960, fig. 3), the bilateral trigeminal arteries have begun retrogression, and dwindle at their carotid origin; they join the bilateral longitudinal neurals near one of several areas where the latter are fused to form the basilar artery. The primitive maxillary artery terminates, as formerly, on the caudoventral aspect of the optic vesicle on the more primitive left side of this embryo. On the right, as in all later embryos, the artery conforms to the lateral margins of Rathke's pouch, proceeding ventromesially to a terminal plexus on the mesial tip of the prosencephalon. The caudal division of the internal carotid has now formed a secondary anastomosis with the

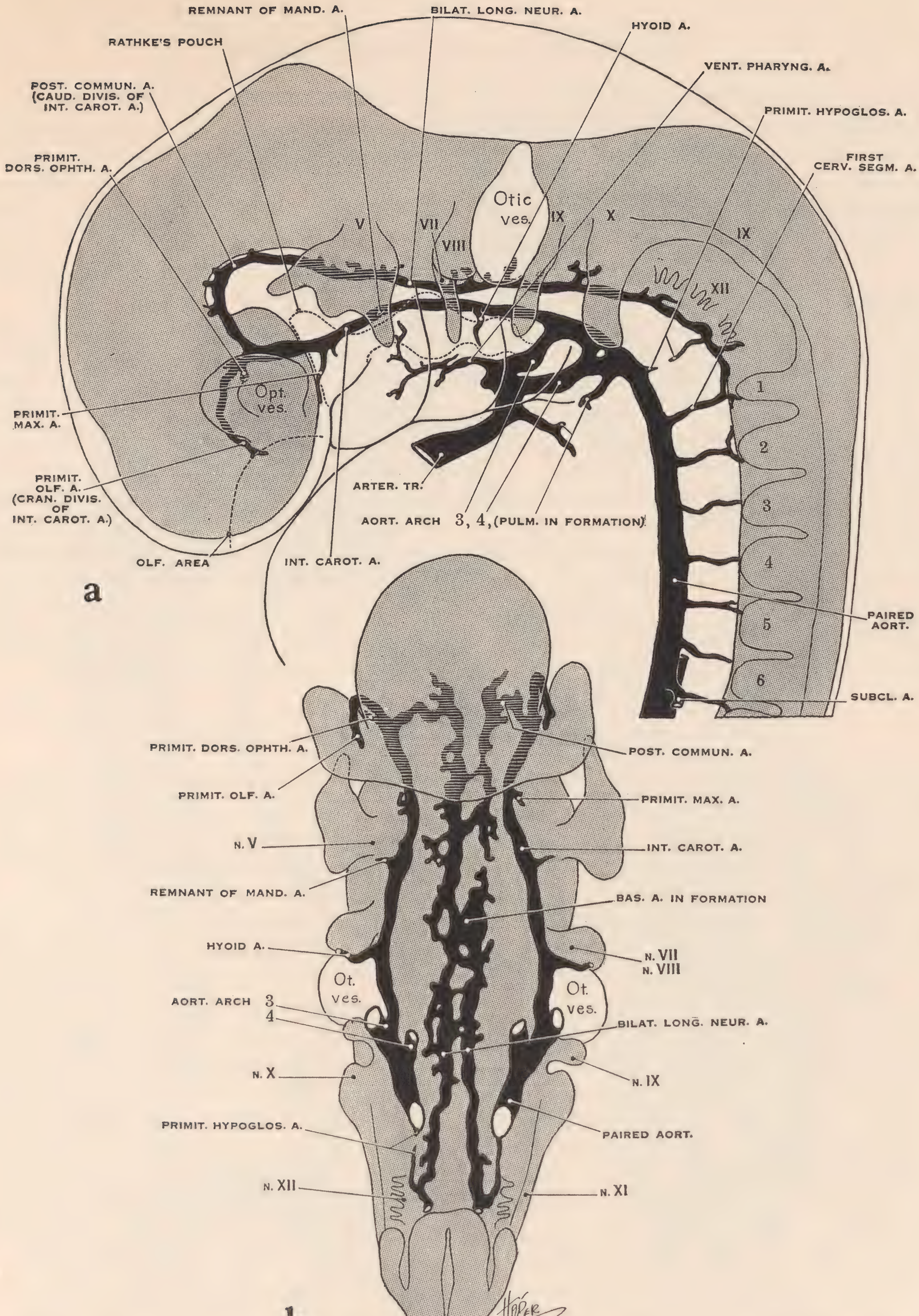


FIG. 2. Stage 2 (see also fig. 3). The cranial arteries in a 5.3-mm. embryo (no. 2841). The caudal division of the internal carotid has joined the bilateral longitudinal neural to form the definitive posterior communicating artery. The primitive trigeminal artery, no longer essential for supply to the cranial end of the neural artery, has disappeared. The basilar artery is being formed by consolidation of the neural arteries (*b*). A ventral pharyngeal artery supplies the greater part of the first two pharyngeal bars. ×37.5.

cranial end of the longitudinal neural artery at the mesencephalon and has thereby become the definitive posterior communicating artery. Soon (fig. 2) this artery entirely replaces the temporary trigeminal artery as the cranial source of blood to the neural or basilar artery. During the preceding stage, one or more twigs of the carotid, near its junction with the posterior communicating artery, passed to the mesial face of the optic vesicle. In the position of these vessels there has developed a relatively long lateral branch which passes over the dorsal and more caudal margin of the developing optic cup to the lens primordium. This is the primitive dorsal (temporal) ophthalmic artery (fig. 3).

The longitudinal neural arteries are not conspicuous in no. 2841 (fig. 2), but are large and packed with primitive red cells in no. 3960. In reference to Congdon's discussion (1922), it is of interest that the latter embryo (fig. 3) gives no evidence that the basilar is being formed from the enlargement of irregular alternating segments of the neural arteries as was long ago described for chick and rabbit embryos (Sterzi, 1904, and De Vriese, 1905, respectively, quoted by Congdon). The process in the human embryo is primarily a matter of fusion according to this embryo. This impression is substantiated by the presence of one or more islands in all parts of the basilar artery (most frequent in the caudal end) in several more advanced embryos up to 21 mm. in length.

The primitive hypoglossal artery persists on one side in no. 2841, but the chief caudal source of blood to the neural artery is the first cervical segmental artery as in the earlier stage. On the hindbrain wall in no. 3960 (fig. 3), between the prominent root masses of the seventh to eleventh nerves laterally, and the sixth to twelfth more mesially, there is a large bilateral channel paralleling the neural artery; this is fed cranially by lateral branches of the basilar, particularly the trigeminal artery, and caudally by the first cervical segmental artery. It is a temporary vessel and, in a later stage, often becomes an accessory anastomosis between the basilar and vertebral arteries.

### Stage 3, Embryos Approximately 7–12 mm., Age Group XVI

The most striking advance of the third stage of arterial development, which brings the "branchial" phase (Congdon, 1922) to a close, is the appearance of first stages in the formation of the vertebral artery (no. 163, fig. 4*a*). The three embryos of different lengths which were fully reconstructed to cover this stage are similar in body form, nervous system, and aortic arches, but illustrate detailed stages in development of several cranial arteries (table 1).

As shown in figure 4, the internal carotid is now characteristically large and swollen in its cerebral part, in contrast with its small stem at the third aortic arch. The hyoid artery is prominent and, owing to shifting and unequal growth changes in the surrounding structures coincident with the ascendancy of the mandibular and hyoid bars over the third or glossopharyngeal bar, its course is caudal, as well as lateral and ventral, to reach its domain in the area between the seventh nerve root, now swung caudally, and the ninth nerve ganglion. In contrast, the mandibular artery either is short and small or is impossible to identify. The ventral pharyngeal artery, whose distal part accompanies the mandibular end of the chorda tympani nerve, supplies the bulk of the first two pharyngeal bars. The primitive maxillary artery, relatively much smaller than before, leaves the internal carotid lateral to the base of Rathke's pouch and courses ventrally. The first notable cerebral branch of the carotid at this stage is the primitive dorsal ophthalmic artery, which now supplies an extensive capillary plexus investing the optic cup. The primary cranial division of the internal carotid gives rise to several branches. The largest of these is the primitive anterior choroidal artery, which supplies the diencephalon in its course to the choroid fissure of the brain. Just distal to this are several twigs representing the middle cerebral stem. Before the cranial division of the carotid terminates at the olfactory pit, it gives off mesial twigs in the path of the future anterior cerebral artery. Opposite the anterior choroidal artery, the carotid supplies a short branch to the more cranial and ventral parts of the optic plexus. This is the primitive ventral (nasal) ophthalmic artery. As will be discussed more fully in a later section of this paper, the arterial supply to the eye remains plexiform until well into the "post-branchial phase" (Congdon), and no single branch of the internal carotid can be traced into the fetal ocular cleft at this stage.

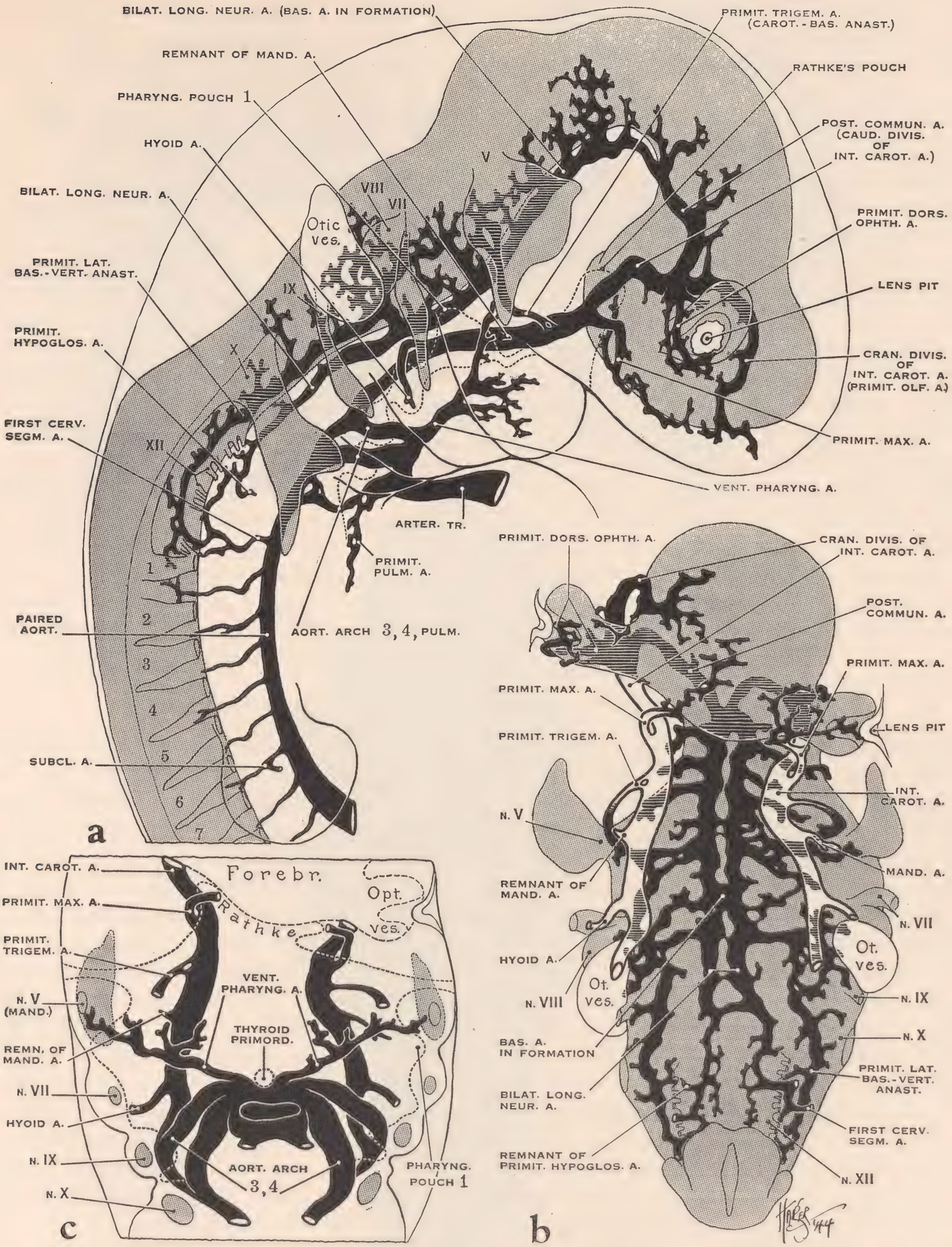


FIG. 3. Stage 2. Right lateral (*a*) and two ventral views (*b* and *c*) of the arteries in a 5.5-mm. embryo (no. 3960) belonging to the same age group as that shown in the preceding figure. Note particularly the persistent primitive trigeminal artery, which is dwindling at the carotid on both sides; the formation of the basilar artery; the large ventral pharyngeal and hyoid arteries, as contrasted with the small remnant of the mandibular artery. Two early stages in arterial supply to the eye are shown on the right and left sides of the embryo, respectively (*b*). ×37.5.

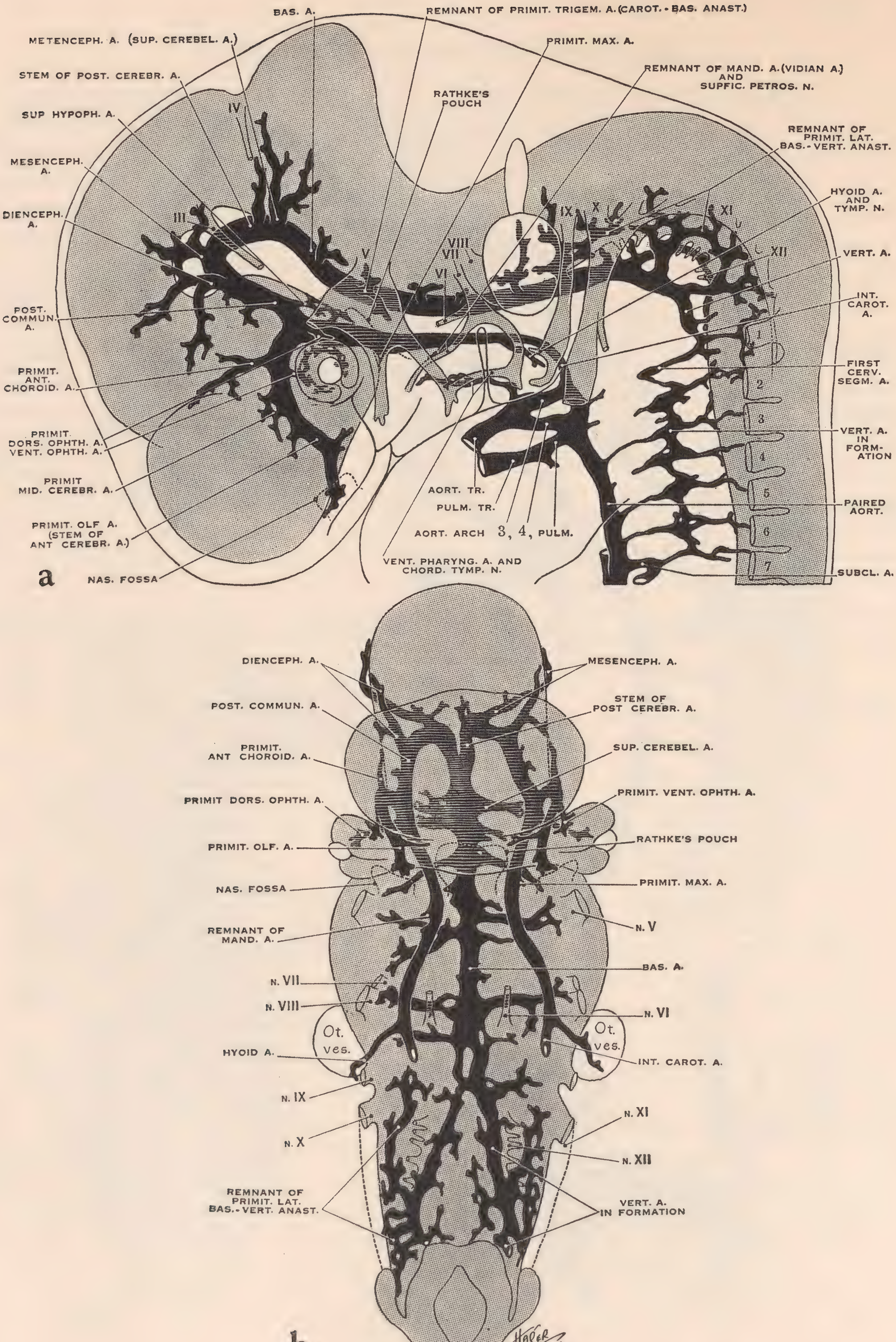


FIG. 4. Stage 3. Lateral view (*a*) of the arteries on the left side of a 9-mm. embryo (no. 163). Note the formation of the vertebral artery from elements of the upper cervical segmentals; the caudal swing of the seventh nerve and its associated hyoid artery; the ventral pharyngeal artery lying against the mandibular end of the chorda tympani nerve; and the two primitive arteries supplying the eye. The primitive olfactory artery (cranial division of the internal carotid) terminates at the nasal fossa. The lower figure (*b*) is a ventral view of a 7-mm. embryo (no. 617) belonging to the same age group as that shown above. ×33.3.

From the caudal end of the posterior communicating artery, just in front of the third nerve, two large branches have emerged. One supplies the diencephalon, and includes a posterior choroidal branch, which is directed toward the domain of the anterior choroidal artery. The other supplies the mesencephalon. The future superior cerebellar artery is now clearly defined, leaving the cranial end of the basilar and supplying the region of the fourth nerve and the developing metencephalon. Caudal to this, the branches of the basilar artery are more or less symmetrical, and are largest at the level of the nerve roots. The most noteworthy feature in the hindbrain region of all these embryos is a lateral channel (or remnants of this channel) paralleling the bilateral longitudinal neural artery as described above in reference to no. 3960 (fig. 3). Now that the basilar and vertebral arteries are almost complete, this channel sometimes constitutes an accessory basilovertebral anastomosis. In its continuous, as contrasted with its partially interrupted, form it is very conspicuous in the 7- and 8-mm. embryos of Elze (1907; see Evans, 1912, fig. 419) and Barniville (1914) respectively. As seen in our embryos, this artery of the hindbrain extends from the level of the otocyst to the first cervical segmental artery, and traverses the area between the sixth and twelfth nerve roots mesially, and the seventh and eleventh roots more laterally.

The basilar artery is irregular and marked by many islands in no. 617, of 7 mm. (fig. 4*b*), but complete in no. 163, of 9 mm. (fig. 4*a*). In the 11.8-mm. embryo (no. 1121; see fig. 45, pl. 5) there are interrupted basilar and carotid remnants of the trigeminal artery which was so conspicuous in the preceding stages; in no. 163 there is a basilar remnant only. The formation of the vertebral artery is well shown in one embryo of this group, no. 163 (fig. 4*a*), where there is a transverse anastomosis between each upper cervical segmental artery and the one adjacent to it. The subsequent obliteration of the proximal or aortal ends of the upper six segmentals is already in progress. As was noted by Congdon (1922), the secondary transverse anastomoses pass caudally from the more proximal end of one segmental artery to the more distal end of the next, so that a significant part of each segmental artery is represented in the completed vertebral, which is zigzag or tortuous in all early stages (compare figs. 4*a* and 5*a*).

### Stage 4, Embryos Approximately 12–14 mm., Age Group XVII

This important stage in general development, which marks the transition from "branchial" to "postbranchial" phase (Congdon), shows several more or less obvious advances in the cerebral arteries. Of considerable interest is the fact that it illustrates a fundamental step in the formation of the stapedial artery. As in the preceding stage, individual differences in correlation of size with nervous and arterial development were apparent, and 4 embryos were reconstructed to determine characteristic features of the group as a whole. There are several inconsistencies in arterial development, one feature being more advanced, another less so in one embryo than in another (see table 1). The description which follows, therefore, is necessarily composite, and two embryos are illustrated in figures 5 and 6.

The hyoid artery, with its carotid stem of origin now at the most cranial level of the hyoid bar, has given rise to an important collateral branch which passes through the stapes primordium between the first pharyngeal pouch and the seventh nerve, and descends into the mandibular substance. Below the pharynx, this secondary branch of the hyoid artery has anastomosed with a distal remnant of the ventral pharyngeal artery which lies on the mesial aspect of the mandibular and chorda tympani nerves (fig. 6; fig. 32, pl. 3). In this manner is established the maxillomandibular division of the now definitive stapedial artery. A component of the external carotid artery, often including thyroid and lingual branches, is present and undoubtedly represents the proximal part of the ventral pharyngeal artery (Congdon, 1922). With the approaching obliteration of the segment of paired aorta between the third and fourth aortic arches (drawn out in a thread in no. 1771, fig. 5), the common carotid artery may now be identified.

Considerable advance is apparent in the arteries supplying the eye (fig. 5). The stem of the primitive dorsal ophthalmic artery is elongated and has two optic branches, a common temporal ciliary artery supplying the caudodorsal aspect of the optic cup, and a hyaloid branch penetrating the fetal ocular cleft. The primitive ventral ophthalmic branch of the inter-

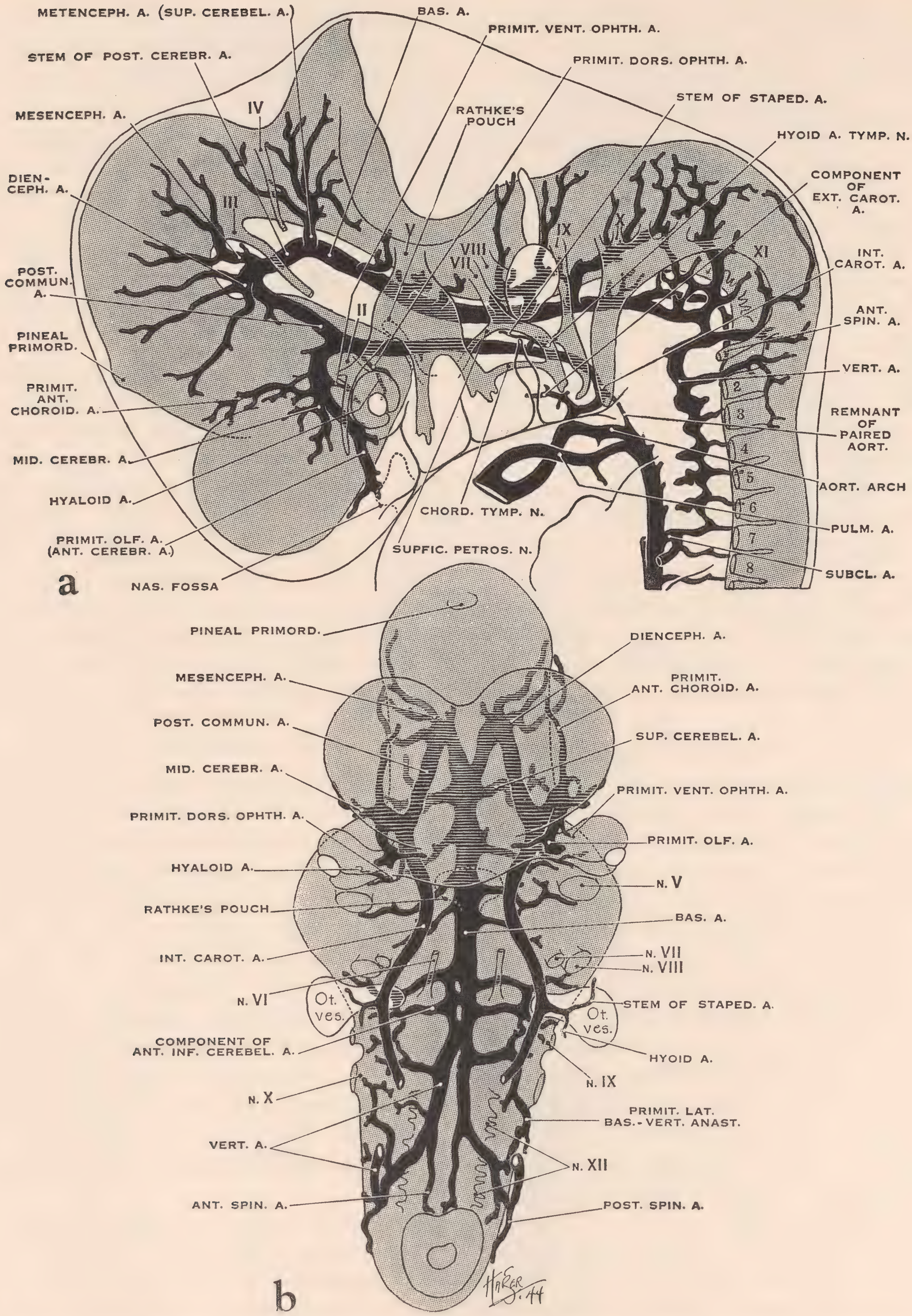


FIG. 5. Stage 4, which ushers in the "postbranchial phase" (see also fig. 6). The cranial arteries in a 12.5-mm. embryo (no. 1771). Developments to be particularly noted are: the new stapedial branch of the hyoid artery, the stem of which has been shifted cranially; the more advanced conformation of the vertebral artery; the emergence of several well defined cerebral arteries; and the elongation and differentiation of the two primitive ophthalmic branches of the internal carotid. ×20.

nal carotid, whose distal part later constitutes the common nasal ciliary artery, is also drawn out in length owing to the beginning elongation of the stalk and ventrolateral shifting of the eye away from the brain wall. It should be noted that the stem of the ophthalmic artery in permanent adult position has not yet appeared.

the situation appears to be the reverse; that is, the middle cerebral appears to be the direct continuation of the carotid artery, with the anterior cerebral as its collateral branch (compare figs. 17 to 21, pl. 1). Another important advance appears at the terminal end of the cranial division of the carotid, which still constitutes a primitive olfactory artery as in all earlier

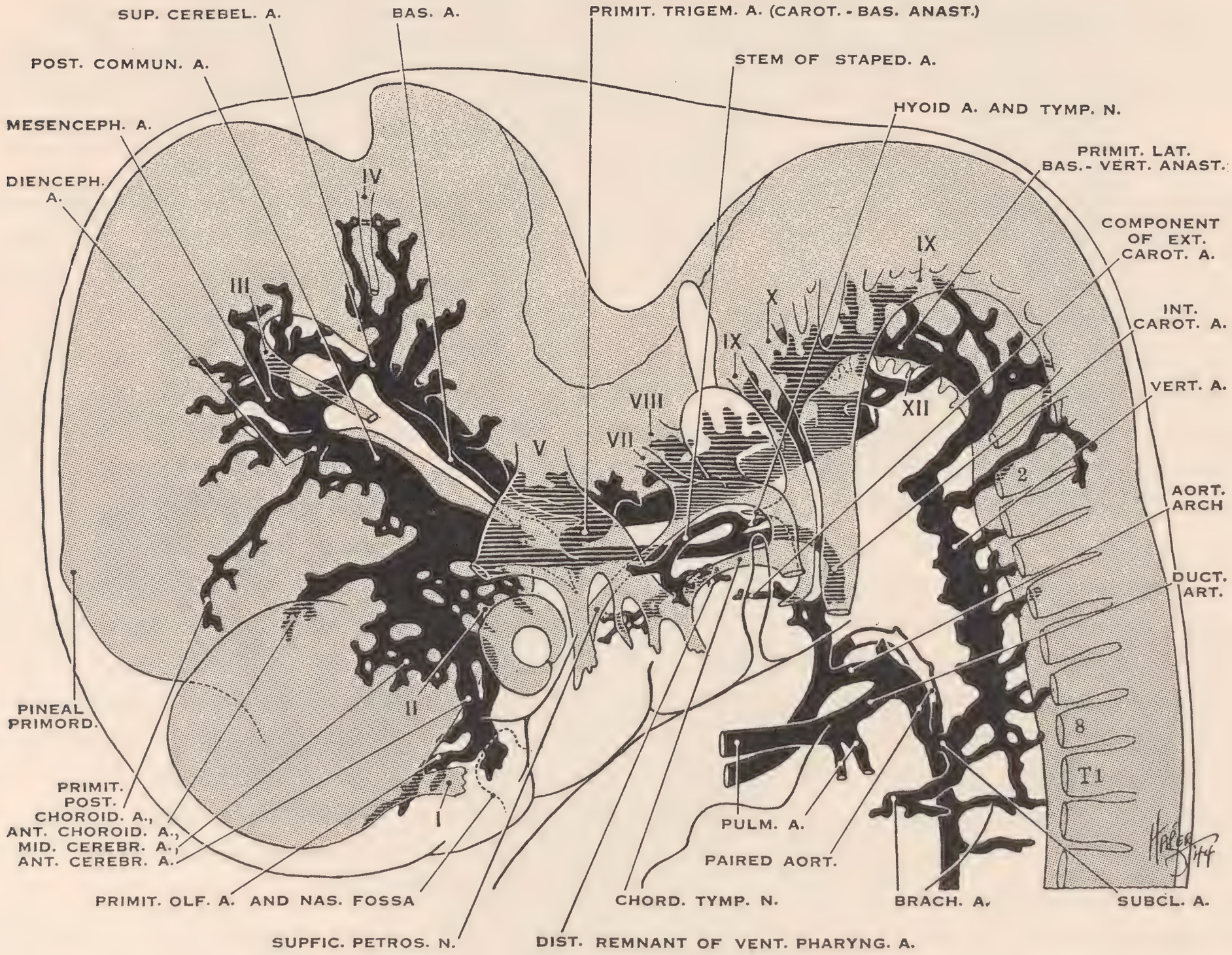


FIG. 6. Stage 4. The cranial arteries on the left side of a 14-mm. embryo (no. 940) belonging to the same age group as that shown in figure 5; although the cerebral arteries are comparatively more primitive—note the persistence of the trigeminal artery (see also fig. 42, pl. 4)—development in general, including that of the aortic arch system, is more advanced. This figure shows the invasion of the mandibular bar by the new stapedial artery, which is a collateral of the hyoid artery. The stapedial has annexed the distal end of the ventral pharyngeal artery, the proximal part of which is now a component of the external carotid. (Details of the stapedial artery supplied from a freehand reconstruction of another 14-mm. embryo, no. 6258.) ×20.

The primary cranial division of the internal carotid may be described for the first time with respect to the definitive parts and collateral branches of the adult conformation. The most proximal segment of this carotid division gives off the anterior choroidal artery as its first branch; the second collateral is the middle cerebral artery, and, with its appearance, the next segment may be named the stem of the anterior cerebral artery. It will be remembered that in the adult stages (figs. 1–5). It now has two branches, the original one to the nasal fossa and a secondary one passing more mesially toward the emerging olfactory nerve root. The latter artery, which represents the future continuation of the anterior cerebral artery, may be joined during the end of this stage with its fellow of the opposite side by plexiform anastomoses in the mid-line region of the future anterior communicating artery (fig. 46, pl. 5).

Further development of the anterior and posterior choroidal arteries, as well as of the basilar and the vertebral arteries with their branches, is readily seen by comparing figure 5 with figure 4 of the preceding stage. Except in two embryos of 11.5 and 14 mm. (see table 1 and fig. 6), the cerebral arteries are far less plexiform and the origin of many adult arteries is already indicated. The trigeminal artery may persist at this stage in the form of a prominent caroticobasilar anastomosis, in which case the posterior communicating artery is small near its junction with the basilar artery (figs. 40–42, pl. 4). The vertebral artery is still irregular, giving evidence of its mode of origin; and in embryo no. 1771 (fig. 5) its cranial part is typically more advanced than its caudal end. This stage is transitional in regard to a significant feature of arterial development in the higher vertebrates, namely, the change from the exclusive carotid supply of all the cerebral arteries to the later substituted supply of the hindbrain region by the vertebrals.

## Stage 5, Embryos Approximately 16–18 mm., Age Groups XVIII, XIX [2]

At this stage, the descent of the heart and large arteries into the thorax is further advanced; the common carotid is thereby elongated, and the vertebral artery, with its subclavian stem of origin now shifted cranially to the adult position opposite the pulmonary arch, is straightened out and fairly regular. With the appearance of the permanent stem of the ophthalmic artery, as well as further differentiation of many other cerebral arteries and external carotid branches, the adult configuration is clearly foreshadowed. Two embryos were reconstructed, and the appearance of the arteries and brain in no. 144 (16 mm., late age group xviii) proved to be almost identical with that in no. 1390 (18 mm., early age group xix), which is shown in figure 7. These embryos are also much like the one of 17.8 mm. described by Thyng (1914) and Bremer (1943) and that of 15.5 mm. (no. 390, age group xix, Carnegie Collection) which was illustrated by Evans (1912, fig. 447).

The stapedial artery is at the height of its development and has two major divisions: the ventral division, which was established in the preceding stage (fig. 6), is the common stem for the mandibular and maxillary (infraorbital) branches; the dorsal or supraorbital division, whose stem appeared in the last stage, now courses lateral to the Gasserian ganglion and far cranially into the primitive orbit. The stem of the ophthalmic artery has finally appeared in adult position, caudal to the optic nerve and lateral to Rathke's pouch, but its domain is limited as formerly to the optic cup and lens. Its three branches are the common temporal ciliary and the hyaloid arteries (branches, during the preceding stage, of the primitive dorsal ophthalmic artery, whose stem has now disappeared) and the common nasal ciliary artery, which represents the distal end of the primitive ventral ophthalmic branch of the internal carotid. At this time, therefore, the components of the future adult ophthalmic artery arise from two widely separated parts of the internal carotid artery, as follows: the orbital arteries come from the most proximal part by means of the supraorbital division of the stapedial artery; the ocular arteries, which temporarily had two separate points of origin from the more distal carotid (the primitive dorsal and ventral ophthalmic arteries), are now branches of the permanent stem of the ophthalmic artery. There is evidence that the stem of the ophthalmic artery has reached its adult position by a caudal "migration" of the primitive dorsal ophthalmic artery along the carotid, effected through a series of anastomotic loops (see page 246).

The fate of two other primitive branches of the internal carotid is indicated at this stage. One may find a small dorsal remnant of the mandibular artery, which accompanies the superficial petrosal (Vidian) nerve skirting the lateral aspect of the carotid. Also seen in some embryos is the small primitive maxillary artery, which is now directed more mesially than ventrally toward the dwindling craniopharyngeal canal along the caudal border of the hypophysis; this becomes a component of the inferior hypophyseal artery. Coincidently with the appearance of the definitive common carotid artery, several external carotid branches may be identified, including the thyroid, lingual, occipital, and external maxillary arteries. The proximal segment of the internal maxillary artery is sometimes seen to extend toward the maxillomandibular division of the stapedial artery, foreshadowing their approaching anastomosis.

This stage shows a noteworthy advance in the ar-

[2] Age group xviii is the oldest group thus far completed in the Carnegie survey. See Streeter (1942, 1945, 1948).

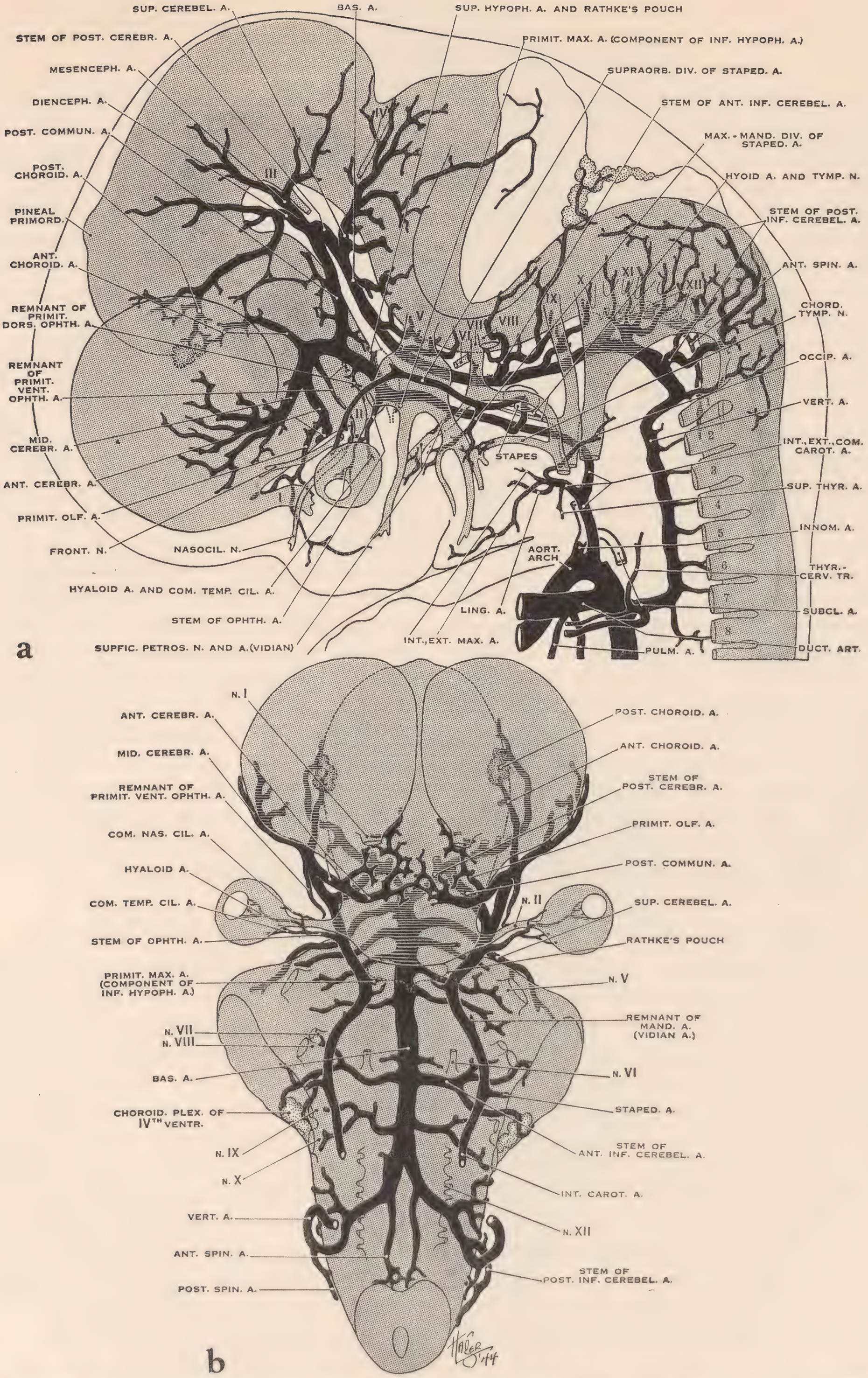


FIG. 7. Stage 5. An 18-mm. embryo (no. 1390) of the period when most of the adult arteries in the head region become recognizable. Note particularly the new adult stem of the ophthalmic artery, which annexes the ocular branches of the primitive ophthalmic arteries (see also fig. 13; fig. 33, pl. 3). The well developed stapedial artery has two major divisions which comprise three branches, mandibular, maxillary, and orbital. ×16.7.

teries of the forebrain. The primitive olfactory artery is still prominent, terminating in the nasal cavity after giving off a large branch to the olfactory nerve. As noted above, however, it has already given rise in the preceding stage to a mesial branch which represents the main part of the anterior cerebral artery. Now the relation of primary to secondary arteries has become reversed, so that the mesial anterior cerebral collateral is topographically the direct continuation of the parent artery, with the more lateral olfactory artery appearing to be its branch.

The middle cerebral artery has become a prominent stem with several branches spreading over the cerebral hemisphere. Both anterior and posterior choroidal arteries terminate in the choroidal infolding at the diencephalic roof. The posterior choroidal artery is the more anteroventral of two large branches supplying the diencephalon, the other coursing toward the pineal primordium. Two equally conspicuous branches supply the mesencephalon. These arteries to the diencephalon and mesencephalon may arise from two stems at the caudal end of the posterior communicating artery just in front of the third nerve, or from a common stem as in no. 1390 (fig. 7). It should be noted here that only the first segment of the future posterior cerebral artery is represented unequivocally in the embryos of this series. De Vriese (1905) emphasized the fact that the proximal end of the posterior cerebral (the so-called "divisional branch of the basilar") is represented early by the most caudal segment of the posterior communicating artery, from which the greater part of the posterior cerebral arises as a collateral relatively late in fetal development. It will be recalled that the adult posterior cerebral gives off several small diencephalic and mesencephalic branches which include the posterior choroidal artery (fig. 11*a*). The developmental emergence of the posterior cerebral artery, through an enlargement of one of this group of branches, when the cerebral hemispheres have begun to envelop the midbrain, may be readily imagined.

The branches of the basilar artery have developed parallel with the differentiation of the metencephalon and the region of the fourth ventricle. The previously established superior cerebellar artery has a mesial division coursing just caudal to the fourth nerve and a lateral one extending over the cerebellar lip. The potential stem of the future anterior inferior cerebellar artery, arising at the eighth nerve level, terminates in the choroid plexus of the fourth ventricle. Caudal to this stem, several branches of the vertebral artery pass between the rootlets of the tenth and eleventh nerves and are frequently joined by anastomoses. One vertebral branch, which is often more prominent than the others and courses cranially along the medulla toward the choroid plexus, may be said to represent the stem of the future posterior inferior cerebellar artery (fig. 7*a*).

### Stage 6, Embryos Approximately 20–24 mm., Age Groups XX, XXI (Provisional) [3]

This stage is a well known one in general craniocerebral development, marking the period when the curvatures of the brain are most pronounced and the head, with recognizably human features, begins to lift away from the chest. These factors, plus the expansion of the cerebral hemispheres, are responsible for the completion of the circle of Willis, which may be more or less readily identified for the first time (fig. 8). The fate of the temporary stapedial artery is revealed as its two major divisions, ventral and dorsal, are annexed by the internal maxillary and ophthalmic arteries respectively. The four reconstructed embryos (table 1) are similar in essentials, and their minor differences emphasize the sequence of events.

[3] See footnote 2.

The internal maxillary branch of the external carotid artery has now established connection with the lower division of the stapedial artery at the junction of its maxillary and mandibular branches. As described by Tandler in 1902 (summarized by Evans, 1912), the internal maxillary branch gains the outer side of the mandibular nerve root by the development of an arterial loop around the nerve with subsequent obliteration of the primary medial limb of the loop. As soon as the common trunk of the maxillomandibular division of the stapedial artery becomes surrounded by the auriculotemporal nerve, the part of this trunk which lies above the recently completed anastomosis with the internal maxillary artery becomes recognizable as the stem of the middle meningeal artery. The supraorbital division of the stapedial artery, in the

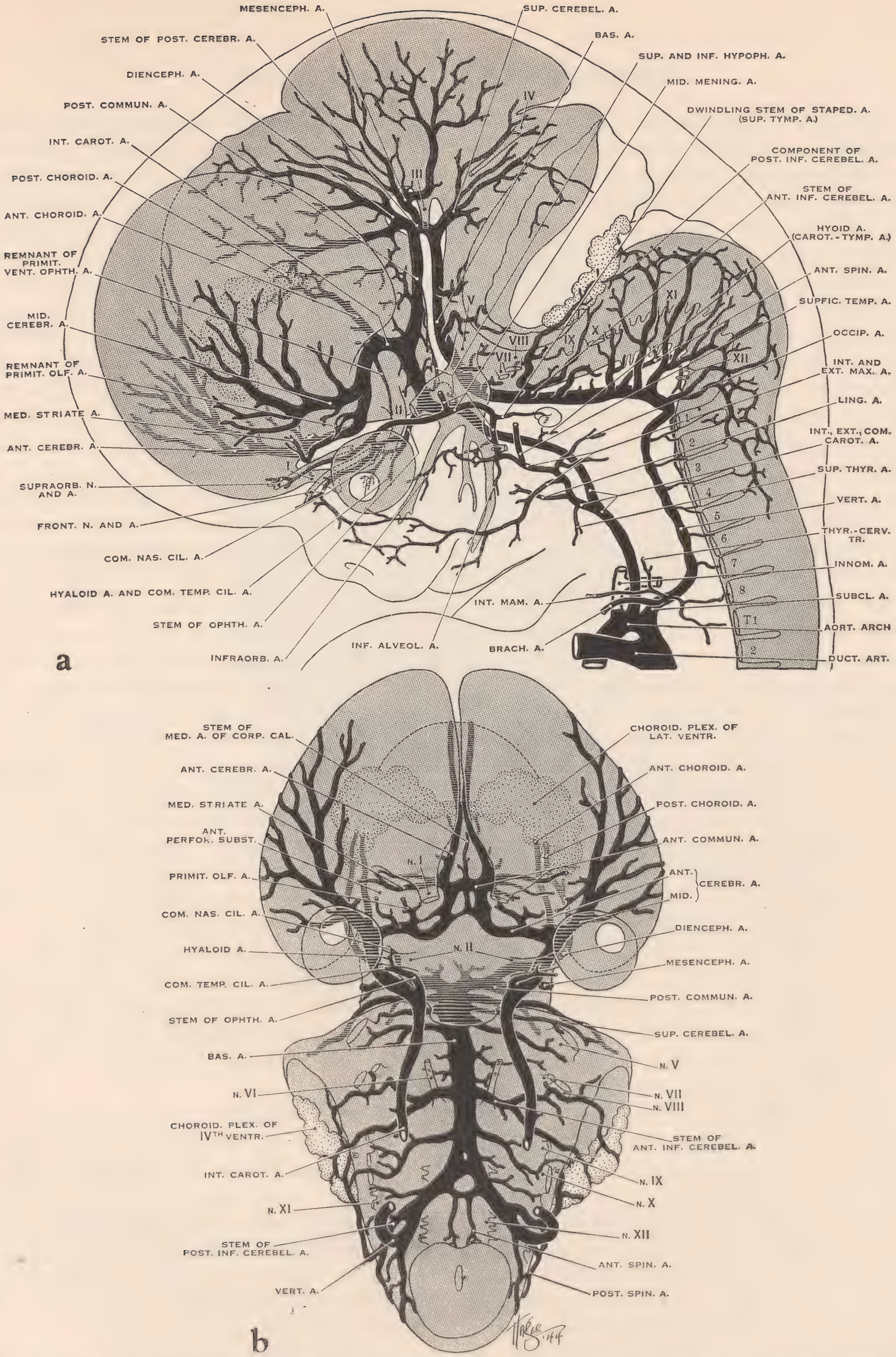


FIG. 8. Stage 6. A 24-mm. embryo (no. 632, injected). Note (*a*) the dwindling stem of the stapedial artery and the two secondary anastomoses by which its major divisions are annexed by, respectively, the ophthalmic artery to form its orbital branches, and the internal maxillary artery to form the stem of the middle meningeal artery. The anterior communicating artery completes the circle of Willis (*b*). Derivatives of the primitive olfactory artery now penetrate the anterior perforated substance as striate branches of the anterior cerebral artery. ×13.3.

part which lies lateral to the Gasserian ganglion, together with a secondary dorsal branch of this artery, represents the extended course of the middle meningeal artery within the primitive dural covering of the brain. In two embryos, the segment of the stapedial artery just mesial to the stapes is dwindling (fig. 9). It is at this point, not at the internal carotid, that the stem of the stapedial artery is interrupted, thereby transposing the two lower stapedial branches to the external carotid artery. The remaining distal part of the interrupted stapedial stem becomes the tympanic branch of the adult middle meningeal artery. The hyoid artery, which, it will be remembered, is the primitive vessel which has just served temporarily as the most proximal part of the stapedial stem, is also retained as the small caroticotympanic branch of the adult internal carotid artery (fig. 8*a*).

The orbital end of the supraorbital division of the stapedial artery has developed two branches, one lateral and one medial, which follow the frontal and nasociliary branches of the ophthalmic nerve, respectively (fig. 34, pl. 3). As noted in the preceding stage, the adult stem of the ophthalmic artery, with its primary common temporal ciliary and hyaloid branches, has annexed the primitive ventral ophthalmic branch of the internal carotid to form its common nasal ciliary branch. This secondary anastomosis, taking place beneath the optic nerve, now constitutes the ventromedial limb of an arterial ring which encircles the nerve (fig. 35, pl. 3). This loop is connected cranially and dorsally with the supraorbital division of the stapedial artery. The means whereby the ophthalmic artery passes from the ventral to the dorsal side of the optic nerve as in the adult, and gains its orbital, as contrasted with its ocular, branches, is now clear. As will be seen, the essential adult configuration of the ophthalmic artery becomes definitive at the next and final stage of this series.

The most important advances in the cerebral arteries concern the region of the anterior cerebral and anterior communicating arteries. In the four embryos examined, the anterior cerebral arteries have approximated each other and course up between the cerebral hemispheres for some distance to supply its mesial surface. It was brought to the writer's attention by Bremer (1943) that the choroid plexus in the region of the foramen of Monro, preceding the development of the corpus callosum from the commissural plate, is supplied by a branch of the anterior cerebral artery. This branch was noted in our injected embryo of 24 mm. (fig. 8*a*, not labeled). In a 39-mm. embryo (no. 6203) of the succeeding stage, this choroidal branch is less direct; since the main stem of the anterior cerebral artery has been drawn farther cranially and dorsally by the expanding hemisphere, its branch loops caudally for some distance before entering the choroid plexus. Shellshear (1930*b*) found in the adult chimpanzee (whose cerebral arteries closely resemble those of man) a fine vessel, continuing the main stem of the anterior cerebral, which recurves around the splenium into the third ventricle and "can be traced as far forward as the foramen of Monro." No example is known of a similar branch in the human adult, and instances of its surviving the long detour imposed by the intervening corpus callosum during embryonic development must be relatively rare.

The anterior communicating artery has not yet formed in one embryo of 21 mm. (fig. 34, pl. 3); it is a single large channel in two others, of 21 and 23 mm. (fig. 9), and is large, but still rather plexiform, in no. 632, of 24 mm. (fig. 35, pl. 3). The completed anterior communicating channel shows in all cases a well marked branch to the commissural plate; the course of the artery is definitely caudal to that of the main stem of the anterior cerebral at this stage (fig. 8*a*, not labeled). De Vriese (1905), conforming with the terminology of earlier writers, called this the "median artery of the corpus callosum" (fig. 8*b*) and found it to be relatively constant though varying in size in the human fetus and adult. This artery may become either an unpaired or an accessory anterior cerebral. In the former instance, found normally in many vertebrates, there is usually an associated lack of development of the paired anterior cerebral arteries.

The fate of the primitive olfactory artery is of considerable interest. The original artery still persists as a small mesial branch accompanying the olfactory nerve into the nasal cavity; but a larger derivative now extends laterally to enter the mesial part of the anterior perforated substance for supply to the developing basal ganglia (fig. 8*b*). Aitken and Ayer (1909) showed that the first segment of the adult anterior cerebral often gives off a small striate branch (or branches). This inconstant branch, varying considerably in size, obviously represents the stem of the

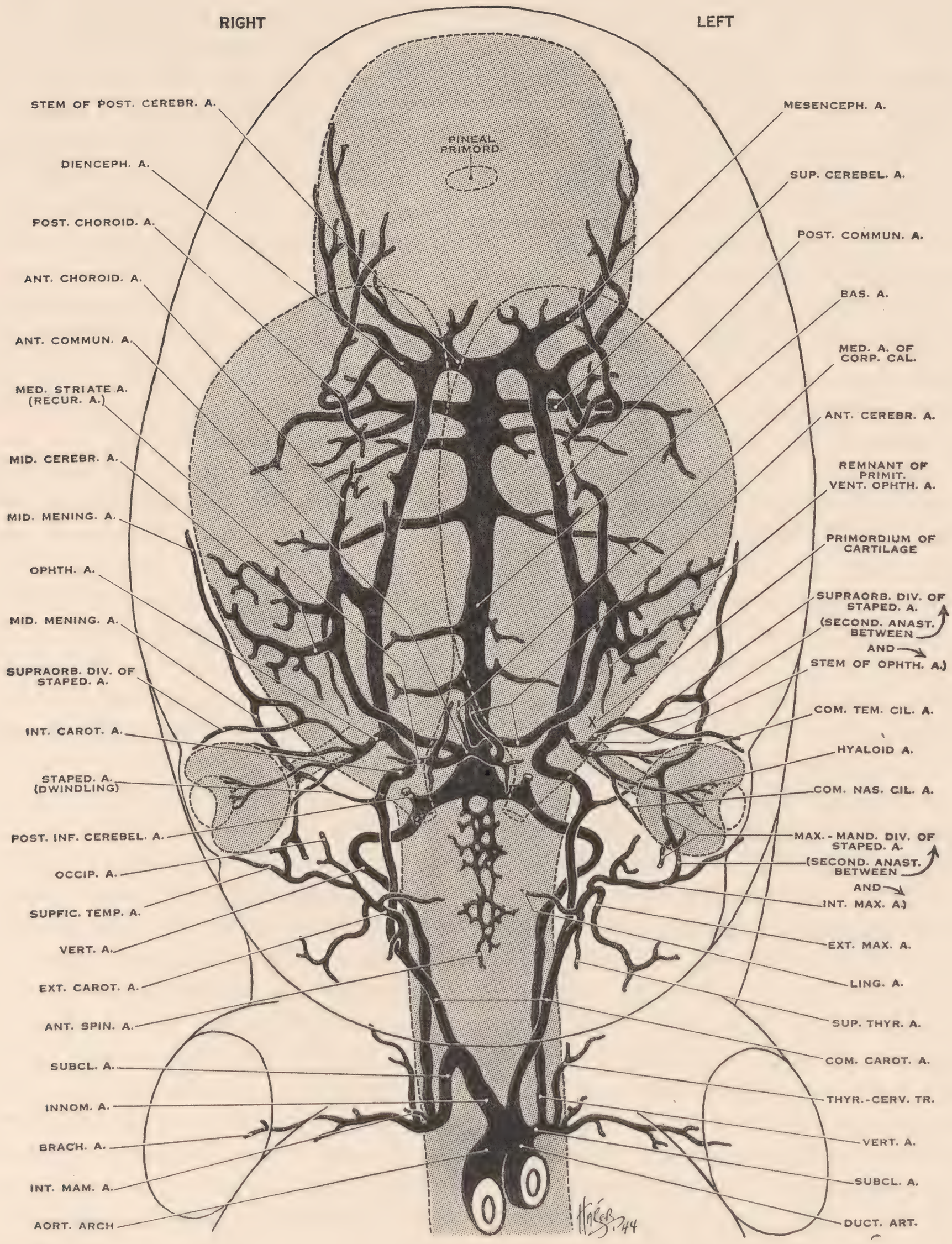


FIG. 9. Stage 6. Coronal (face view) reconstruction of a 23-mm. embryo (no. 966) of the same age group as that shown in the preceding figure. The recently completed circle of Willis can usually be seen only from this point of view, owing to the extreme cerebral curvatures of the 20-mm. stage (compare fig. 8). The two secondary anastomoses formed by branches of the stapedial artery (its stem interrupted in the preceding embryo and dwindling here) with the stem of the ophthalmic artery and with the internal maxillary artery are well shown. ×16.7.

primitive olfactory artery (fig. 7), that is, the terminal part of the cranial division of the primitive internal carotid, seen in the youngest embryos of this series (figs. 1, 2). These authors were the first to describe comprehensively another central or ganglionic branch of the anterior cerebral, which is relatively constant and which they called "Heubner's artery"; this was noted by Abbie (1934, 1937) to be "phylogenetically one of the oldest of cerebral arteries." As confirmed by others (Critchley, 1930, who discusses its clinical significance), this has also been called the "recurrent branch of the anterior cerebral" (Shellshear, 1920, 1930*a*, 1930*b*) or the "recurrent artery of Heubner" in reference to its recurrent course laterally along the anterior cerebral artery to the anterior perforated substance. Abbie (1937) suggested the definitive term "medial striate artery," which may also be applied properly to the less constant remnant of the primitive olfactory artery just described. Among leading textbooks of anatomy, only the 1944 edition of Gray's *Anatomy* and Strong and Elwyn's 1943 *Neuroanatomy* mention this artery which, although variable in size, point of origin, and number of branches, supplies the more anteroinferior parts of the corpus striatum and internal capsule. It was noted by Alexander (1942) that the central branches of the adult anterior cerebral are developed reciprocally with those of the middle cerebral artery; in the 20- to 40-mm. embryos (figs. 8, 10) the former are far more conspicuous. The medial striate or recurrent artery of Heubner is formed from components of the anastomosis between the primitive olfactory artery and its collateral anterior cerebral branch, seen in earlier embryos (fig. 34, pl. 3; compare figs. 6, 7, 8). It leaves the anterior cerebral near or opposite the anterior communicating artery and extends laterally for some distance between the olfactory lobe and the brain before entering the anterior perforated substance (figs. 8–11).

With the formation of the anterior communicating artery, the circle of Willis is completed. It is, however, seldom readily recognizable in the ventral or base view of the embryo, because of the exaggerated cerebral curvatures of the 20-mm. stage. The posterior communicating artery, which at first proceeded cranially from the carotid to the basilar artery and appeared to be a direct continuation of the carotid, is gradually swung upward and finally backward as on a hinge, and ultimately leaves the carotid at an angle of approximately 90°. At the 20-mm. stage, when the posterior communicating artery extends dorsally upward, the recently formed circle of Willis can often be demonstrated only by a coronal (or face) view of the specimen. As thus seen, its configuration is confusing unless all the topographical relations are kept in mind (see fig. 9, which is obviously the same view as the "arteries at the base of the skull and eye" in a 20-mm. embryo reproduced from Versari in the Keibel and Mall *Manual,* vol. 2, fig. 180, 1912).

As in preceding stages, the anterior and posterior choroidal arteries are connected by anastomoses over the diencephalon in the region of the choroid plexus, a condition retained to a greater or less extent in the adult (Beevor, 1909; Abbie, 1933). It was shown by these authors and emphasized by Alexander (1942) that supply to the choroidal plexus by the adult anterior choroidal is subordinate to its extensive central distribution, for instance, to the greater part of the globus pallidus and posterior limb of the internal capsule, the optic tract, the tail of the caudate, and the amygdaloid nucleus. Cortical branches supply the uncinate gyrus. Alexander was disposed to rename the anterior choroidal the "pallido-hippocampo-capsular artery."

At this late stage, the stems for both anterior and posterior inferior cerebellar arteries, especially the latter, may still be difficult to identify among the numerous arteries supplying the posterior part of the hindbrain. The plexiform conformation persisting relatively late in this region is in keeping with the fact that the cerebellar lobes have not yet formed. Wide variations in the origin and course of the branches of the basilar and vertebral arteries in this region have been described for the adult by Stopford (1916). These are explained chiefly by the late retention of different remnants of the lateral basilovertebral anastomosis (see p. 214). A 12-mm. embryo (fig. 5*b*) of stage 4, when the point of emergence of the sixth nerve from the hindbrain is well caudal to that of the seventh and eighth nerves, explains the origin of one type of variation. (A duplicate of this arterial configuration was shown for the adult echidna by Shellshear, 1930*a*, his fig. 1.) Here the sixth nerve is bounded by a closed vascular ring composed of the following: mesially, the basilar artery; cranially, a basilar branch passing dorsal to the nerve and terminating at the

seventh and eighth nerves just ahead of the otocyst; caudally, another basilar branch to the ninth nerve behind the otocyst; and laterally, an anastomotic channel between these two basilar branches which represents a part of the primitive lateral channel mentioned above. If the lateral anastomosis is obliterated, the internal auditory and anterior inferior cerebellar arteries arise separately from the basilar, as shown in figures 10*b* and 11*b* (left side). Loss of the cranial segment is the most common outcome, owing to the cranial shift of the abducens in relation to the facial-acoustic nerves and the fact that the arteries are attracted caudally by the enveloping cerebellum. This leaves the internal auditory artery as a branch of the anterior inferior cerebellar (62 per cent of cases, Stopford) which passes ventral to the sixth nerve (about 75 per cent, Stopford) and is shown in figures 7*b*, 8*b*, and 11*b* (right side). The less frequent instances in which the anterior inferior cerebellar and its auditory branch pass dorsal to the nerve represent a loss of the caudal segment of the original vascular ring or, as Shellshear expressed it, a retention of the original internal auditory artery, of which the anterior inferior cerebellar is a collateral. It was reported by Cushing (1910) that the sixth nerve, in cases of brain tumor in this region, is often strangulated by the anterior inferior cerebellar artery when the artery lies ventral to the nerve.

### Stage 7, Embryos of Approximately 40 mm. and Adult Configuration

In the final stage of this series, the adult stem of origin of essentially all the head arteries can be readily identified. The relative size and direction of many as well as the termination of others, notably the posterior cerebral and both inferior cerebellar arteries, remain to be determined by the predominance of the cerebral and cerebellar hemispheres over the brain stem and medulla respectively. Two embryos of different lengths, but otherwise similar, were graphically reconstructed; no. 886 (43 mm.) illustrates general characteristics, and certain important details, particularly of the ophthalmic artery, were supplied by no. 6203 (39 mm.).

Figure 10, as compared with the preceding embryonic stage (fig. 8) and with the adult form shown in figure 11 (see also pls. 1, 2), shows the arterial advances which are in keeping with the development of the brain and head. The circle of Willis is readily seen for the first time in the ventral or base view of the brain, owing to the caudal swing of the posterior communicating artery. Note the large size of this artery, which is characteristic not only of the embryo, but also of the newborn (over half of a hundred cases, according to De Vriese, 1905). Considerable asymmetry and variation in size is often encountered in the posterior communicating artery of the newborn and adult, and also in the divisional branch of the basilar (that is, the first part of the adult posterior cerebral, representing the original caudal end of the embryonic posterior communicating artery, which terminates in the basilar; fig. 11*b*). The posterior cerebral is not infrequently given off from the internal carotid in the position of the posterior communicating artery, a variation often accompanied by small size of the connecting divisional branch of the basilar. The typical adult posterior communicating artery is small, of a caliber a little larger than that of the anterior choroidal artery, according to De Vriese. The diminished size of the vessel is the result of the transfer of the blood flow of its posterior cerebral collateral, which forms late, from the carotid to the basilar and vertebral system.

Figure 11, showing the cranial arteries in the newborn, illustrates aspects of adult configuration which are inadequately or incorrectly depicted and described in many textbooks of anatomy. Note particularly (11*b*) that the origin of the anterior cerebral, which is usually dorsal in the adult and, therefore, hidden from below by the parent internal carotid, is just distal to the anterior choroidal artery, although many current illustrations show it arising opposite or even proximal to the posterior communicating artery. The median artery of the corpus callosum, which is often well developed at term (De Vriese, 1905), sometimes constitutes an accessory or median anterior cerebral. The greater part of the relatively constant medial striate artery is usually hidden by the parent anterior cerebral when the adult circle of Willis is viewed *in situ*. The superior hypophyseal arteries, which are ordinarily very small, are exaggerated in the accompanying illustration, and the inferior hypophyseals from the intracavernous part of the internal carotid are not shown. Note the variable origin of the two inferior cerebellar arteries, which are formed much

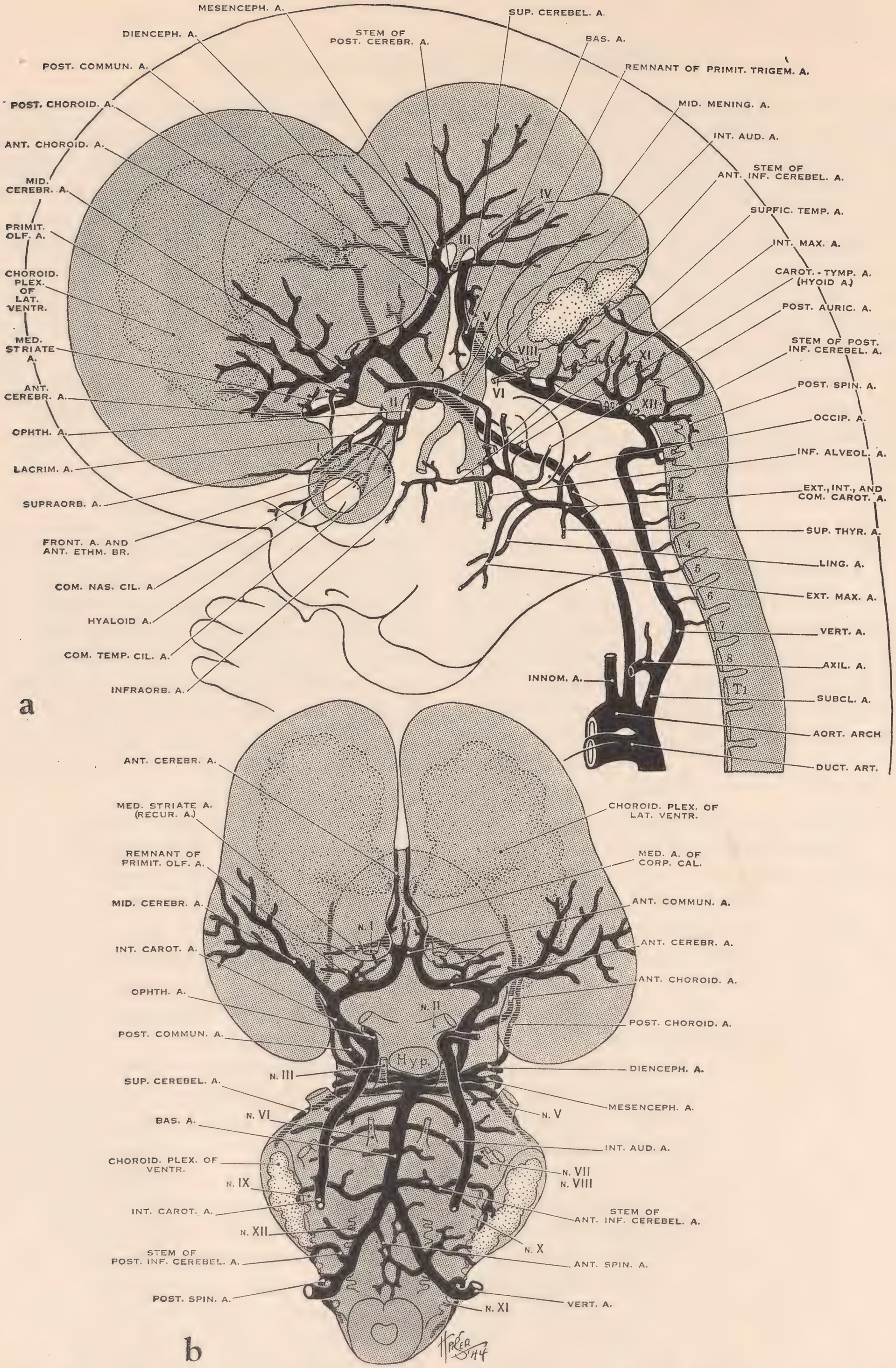


FIG. 10. Stage 7. A 43-mm. embryo (no. 886); details supplemented by reconstructions of no. 6203, 39 mm. Although the embryos are less than two months old, their cranial arteries are readily comparable to those of the adult. Further extension of the posterior cerebral and both inferior cerebellar arteries awaits only the future predominance of the cerebral and cerebellar hemispheres. The ophthalmic artery is complete, having incorporated part of the supraorbital division of the stapedial artery as its lacrimal branch. The definitive middle meningeal artery and essentially all external carotid branches are present. ×6.7.

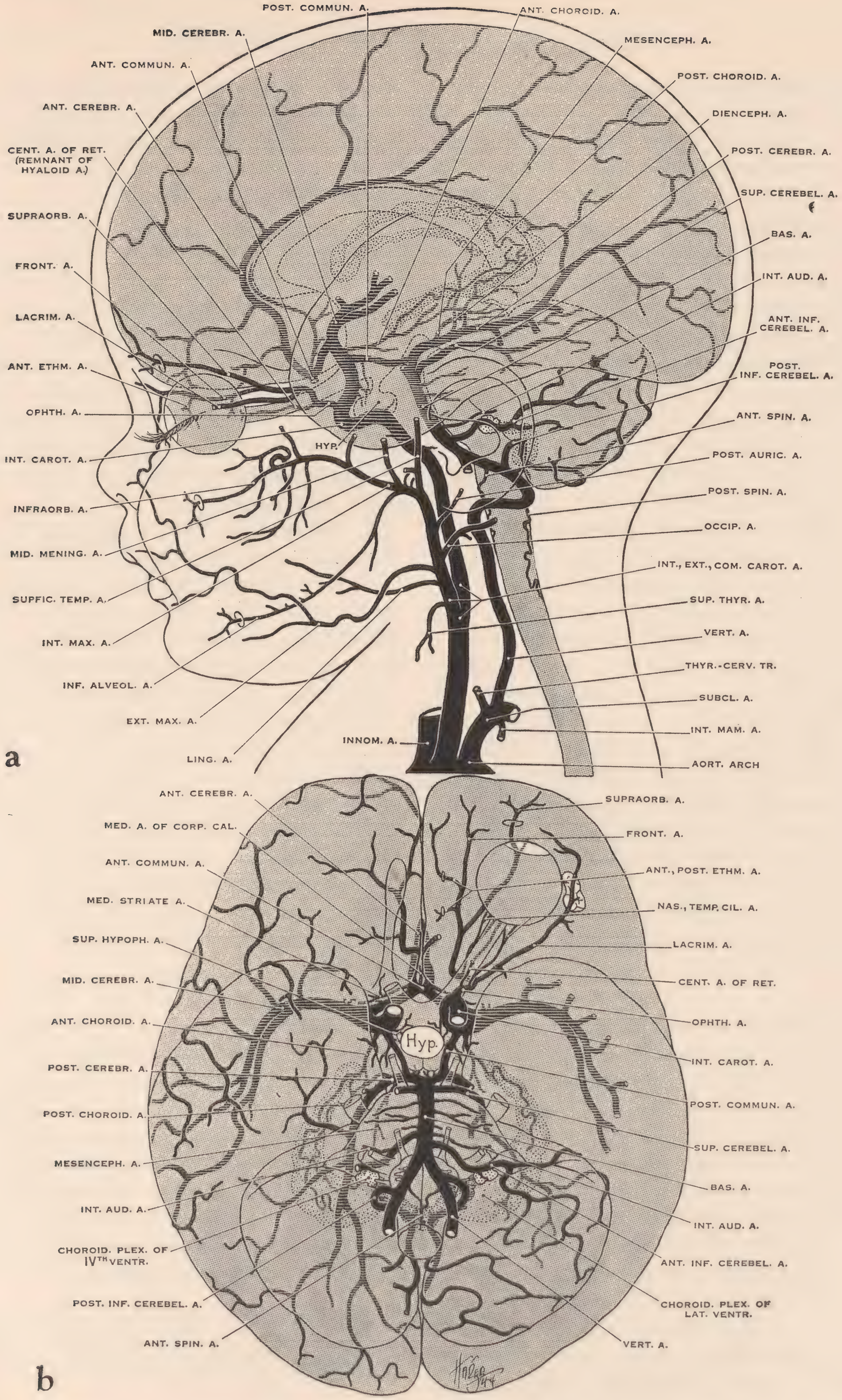


FIG. 11. The newborn configuration, which is essentially the same as that of the adult, although there is typically less relative difference in the size of the arteries composing the circle of Willis and those immediately derived from it. See page 227 for the details to be noted in this figure.

later than the superior cerebellar, whose position is relatively constant (11*b*). The posterior choroidal branch (or branches) of the posterior cerebral commonly anastomoses with the anterior choroidal, and is more significant in the supply of the choroid plexus of the lateral ventricle than is the anterior choroidal, which is an important central or ganglionic branch of the internal carotid. The small caroticotympanic branch (embryonic hyoid artery) and, above it, the "Vidian" branch (embryonic mandibular artery) of the petrosal internal carotid are shown but not labeled in figure 11*a* (compare figs. 7, 8, 10).

The ophthalmic artery has attained the approximate adult conformation (fig. 11*a, b*) in the 40-mm. stage (fig. 10*a*), during which time there is either a loss or a dwindling of its connections with the temporary stapedial artery, whose stem was interrupted in the preceding stage (fig. 8). The lacrimal artery has completed the orbital branches of the ophthalmic artery, and its proximal end represents the strictly orbital part of the supraorbital division of the stapedial artery (figs. 35, 36, pl. 3). In the preceding stage, the proximal (extraorbital) part of this stapedial division became incorporated in the middle meningeal artery. Now, a more distal segment of this division, between the middle meningeal and ophthalmic (lacrimal) arteries, has been interrupted just outside the orbit. As noted by many anatomists, an anastomosis of variable size between the lacrimal branch of the ophthalmic and the anterior branch of the middle meningeal arteries is often found in the adult; sometimes the latter artery gives origin to many or even most of the branches of the former. This is a persistence of the embryonic stage at which the upper division of the stapedial, before its complete annexation by the ophthalmic artery, is the chief supply of the orbit. It is also of interest that the right optic nerve of one embryo of the 40-mm. stage (fig. 36, pl. 3) is still surrounded by the arterial circle which formed in the preceding stage (fig. 8). On the other side of this embryo, a cranioventral segment of this circle, actually the root portion of the secondarily formed common nasal ciliary artery, has disappeared. This leaves the ophthalmic artery readily comparable to the typical adult form, with the exception of the hyaloid artery, which dwindles later on in fetal life, and is represented in the adult by the central artery of the retina (fig. 11*a*).

In conclusion, attention is called to the detailed study by Beevor (1909) on the distribution of the adult cerebral arteries (excluding the branches of the basilar and vertebral arteries, fully reported by Stopford in 1916). He injected several arteries simultaneously—in some cases as many as five, the anterior, middle, posterior cerebrals, the posterior communicating, and anterior choroidal—under the same gradual pressure with gelatine containing soluble colors which filled the finest capillaries without transuding through the tissues. His study is based on approximately a hundred brains and is illustrated with colored photographs of representative brain sections. Details concerning the configuration and supply of several arteries reported since, many of them referred to above, have essentially corroborated all of Beevor's findings.

## THE STAPEDIAL ARTERY

Tandler (1902) reported in detail on the development of the stapedial artery in the rat, and described certain late stages in the human embryo. He showed how the stem of the stapedial is taken over by the external carotid, including its role in the formation of the middle meningeal artery, but failed to reveal how it is formed in man. Fuchs (1905), in an exhaustive study of orbital vascular development in the rabbit, also dealt briefly with the stapedial artery, including early stages. Furthermore, he showed the part played by its supraorbital division in the formation of the orbital arteries in this species. Congdon (1922) was not concerned with the development of the stapedial artery, but discussed the relation of the second aortic arch to its stem of origin and pointed out that only especially well injected specimens can be relied upon for determining the changes in vascularization connected with the involution of the first two aortic arches. The relatively large number of embryos available for the present study, particularly as revealed by graphic reconstruction,[4] has clarified the factors leading to the formation of the stapedial artery and has also demonstrated its role in the development of the ophthalmic artery. The stapedial

[4] Vascular changes in the background of formation of the stapedial artery are dependent on several relevant changes in the whole embryonic head, which could not be demonstrated within the limitations imposed by construction of a model.

artery is retained prominently in the adult rat, as is generally known through Evans' review (1912). A fundamental difference in the evolution of the artery in the rabbit was found to resemble closely that in man. In both species the stapedial is a temporary embryonic vessel.

## Development of the Stapedial Artery in Certain Vertebrates

The stapedial artery is of considerable interest as an instance of supply to one region of the young embryo head, the mandibular bar (arch) and parts cranial to it, by an artery belonging to another region, namely, the well defined hyoid bar. This phenomenon, which takes place during the emergence from the branchial period, is described in simplest terms by Tandler (1902) for the rat. By the time the third, fourth, and pulmonary aortic arches are completed in the 3.5-mm. rat embryo, the second has already dwindled and the first is interrupted, though prominent vessels representing its dorsal and ventral ends persist. The dorsal remnant of arch I [5] (mandibular artery) traverses the mandibular bar, after giving rise to a branch passing over the oral cavity into the maxillary process. In embryos of 5 mm., a supraorbital branch has also been added to the dorsal remnant of the first arch. The second aortic arch is now interrupted, and its dorsal remnant (hyoid artery) has assumed a deviated caudoventral course behind the first pharyngeal pouch. Since the position of the developing stapes primordium is somewhat cranial and dorsal to the first pharyngeal pouch, it is obvious that the hyoid artery can supply it only through a secondary branch. This stapedial collateral has appeared in the 6.5-mm. embryo and passes cranially to anastomose with the primary mandibular branch of the dorsal remnant of the first arch. The model in Tandler's plate 4, figure 13 shows the dorsal remnant of arch II (hyoid artery) and its recent stapedial branch in their respective positions, caudal and cranial to the first pharyngeal pouch, a configuration which is essentially that of an analogous stage in the embryos of both rabbit and man.

The dorsal end of the first (mandibular) aortic arch is obliterated proximal to its three branches in a subsequent stage which completes their annexation by the stapedial branch of the second (hyoid) arch. Eventually, the mandibular branch of the stapedial artery is largely taken over by the external carotid artery through another secondary anastomosis. The stapedial artery persists, however, in the adult rat as a prominent branch of the internal carotid, retaining its maxillary and supraorbital branches and also contributing to its former mandibular branch.

In the rabbit, according to Fuchs (1905), the stem of the stapedial artery, as in the rat, arises as a secondary branch from the dorsal remnant of the second aortic arch (hyoid artery) and passes forward between the tympanic cavity and the seventh nerve into the mandibular bar. In marked contrast with the situation in the rat, however, the remnant of the dorsal end of the first aortic arch (mandibular artery) has dwindled without having given origin to any branches. At the time of its obliteration, the mandibular and hyoid bars are largely supplied by what Fuchs called the "external carotid" or "ventral remnant of the first two aortic arches." This vessel, as described and illustrated in sections of the rabbit embryo, is definitely homologous to Congdon's "ventral pharyngeal artery" in man. The so-called external carotid of this stage in the rabbit gives off a sizable branch ("Oberkieferast"; see Fuchs' figs. 32, 33, pls. 11, 12) into the lateral maxillary region, and it is with this vessel that the newly formed stem of the stapedial artery makes an anastomosis. As Fuchs expressed his findings in contrasting the rabbit with the rat, the stapedial gains its branches not by annexation of a dorsal "rest" of the first aortic arch, but from "peripheral branches of the external carotid," representing ventral remnants of the first two arches. Although Congdon's discussion has modified the terminology and interpretation of the arteries involved, this manner of formation of the stapedial artery was found to be essentially that of the human embryo.

Unlike that in the rat, the supraorbital branch of the stapedial artery in the rabbit arises, not in common with the other two stapedial branches, but as a derivative of the maxillary branch ("Oberkieferast"). This "bulbo-orbital" artery gains an important role in the rabbit, giving origin to all the orbital arteries and largely annexing even the ocular branches of the

[5] Although Congdon (1922) argued against the advisability of use of the term "arch" for the vessels seen during the involution of the first two aortic arches, the nomenclature of the writers quoted is used in these reviews, with revised terminology in parentheses.

internal ophthalmic artery with the exception of the central artery of the retina. Ultimately, the three branches of the stapedial artery are all taken over by the external carotid, and the stem of the stapedial is interrupted near its origin from the internal carotid.

Grosser, cited by Tandler, Fuchs, and Evans, refers to a variation found in a bat embryo (fig. 34 of Tandler, 1902) in which the stapedial artery gives rise to only mandibular and supraorbital branches. An infraorbital (maxillary) branch, identified as a dorsal remnant of aortic arch I (mandibular artery), is present as a separate derivative of the internal carotid artery. It arises cranial to the stem of the stapedial artery, at the point where the carotid is in contact with the superficial petrosal (Vidian) nerve and follows the nerve to reach the maxillary branch of the fifth nerve. This so-called "Vidian artery" occurs in many adult reptiles, according to Evans, and is of interest in connection with observations on the human embryo to be described below.

## Formation of the Stapedial Artery and Its Branches in Man

It is suggested by Evans (1912) that "in man the three branches of the stapedial artery, instead of being derived from the dorsal end of the first [aortic] arch [as in the rat], are probably derivatives of the ventral portion of that arch." This study essentially bears out his statement. Congdon (1922) claimed that the "arch" components Tandler described as taking part in stapedial formation were to be regarded not as late stages of, but rather as vascular successors to, the first two aortic arches. He described "two pairs of successive vessels," stating that "during the branchial period they serve to supply the substance of the corresponding branchial [pharyngeal] arches" and named them respectively the "earlier" and "later mandibular and hyoid arteries." In the present study a prominent and relatively long hyoid artery was observed constantly until and beyond the time of development of its stapedial branch; but the mandibular artery, with the frequent exception of a small dorsal remnant, dwindles early. Particular attention, therefore, was directed to the question of the blood supply to the mandibular and maxillary regions following the involution of the first aortic arch.

The evidence on which Congdon based his description of "successive channels" consisting of earlier and later mandibular and hyoid arteries is not altogether clear. According to him the "earlier" arteries course near the axis of their corresponding pharyngeal bars and are replaced by the "later" close to the caudal border of each bar; "all break up in a plexus in the subpharyngeal region" and "their current is from the dorsal aorta." Our graphic reconstructions of embryos, including two modeled for Congdon's study, show that the course of each earlier artery is related to the more caudal border of the pharyngeal bar. The terminal part of the long hyoid artery retains this position, but eventually that of the short mandibular artery is swung more cranially. What is more important, the carotid origin of each artery appears to move to the cranial, rather than the caudal, edge of the corresponding bar. In two 4-mm. embryos (no. 836, fig. 39, pl. 4; no. 588, fig. 1), the earlier mandibular and hyoid arteries, identified by Congdon, are relatively large and more or less continuous from dorsal to ventral ends, although branched and somewhat plexiform; here each may be said to supply the substance of its related bar, although not necessarily from the dorsal direction. However, in a 5.7-mm. embryo (no. 1380, reconstructed by Congdon and also for the present study), which is more advanced, only dorsal ends are found, and that of the mandibular artery is reduced to a mere fragment. Certainly the later mandibular artery, which was identified by Congdon in this embryo, does not appear to supply its related bar, since it can be traced for only an insignificant distance laterally from the carotid. Since no evidence was found in the present study for other than retrogression or differentiation, respectively, of the original mandibular or hyoid arteries which succeed the first two aortic arches, the designation "earlier" and "later" will be omitted. The embryos on which the comments above are based will now be described in more detail.

The two 4-mm. embryos (no. 836, figs. 38, 39, pl. 4; no. 588, fig. 1) just mentioned show successive stages in arterial development, the fourth aortic arch being in beginning formation in the first specimen and fully developed in the second. A tortuous plexiform mandibular artery has now replaced the first aortic arch. Its dorsal end dwindles immediately, and its initial course is not only lateral but also caudal, before plexiform connections join it with the lateral and

ventral parts of the same artery in the body of the mandibular bar. This marked caudal deflection in course appears to be dependent upon the oral groove now partly dividing the first pharyngeal bar into definite maxillary and mandibular regions. The most dorsal and lateral parts of the artery are so small and plexiform that its continuity in this region is highly questionable. On the left side of embryo no. 836, the dorsolateral part of this primitive mandibular artery appears to give off small branches which pass over the oral cavity into the emerging maxillary process (fig. 38, pl. 4).

In two more advanced embryos (nos. 1380 and 2841), in which the pulmonary arch is forming, the irregular hyoid artery, which has replaced the second aortic arch of the earlier embryo (no. 588, fig. 1), is definitely interrupted, but the remaining dorsal end is relatively long and extends not only laterally but also ventrally for some distance before dwindling (fig. 2). The mandibular artery is now difficult to identify, there being only a short dorsal remnant.[6] It was not possible to trace any vessels in the mandibular substance of no. 1380; but in no. 2841, which also shows the small dorsal fragment of the mandibular artery almost immediately breaking up into capillaries, there are fairly well defined arteries in the subpharyngeal region. These arise from the aortic sac as irregular bilateral channels in the general position of the ventral ends of the first two aortic arches. Each passes cranially and laterally through the hyoid into the mandibular bar and is lost in a plexus around the mesial border of the mandibular branch of the fifth nerve (fig. 2). Congdon (1922, pl. 2, fig. 35) labeled this the ventral-pharyngeal artery, stating that the vessel originally borders the thyroid primordium, is gradually withdrawn from the mid-line or replaced by a more lateral vessel during the "branchial" period, and finally constitutes a component of the external carotid artery (figs. 30–32, pl. 3).

Other embryos of this period were studied to verify, if possible, the arterial pattern at times equivocally revealed by the four embryos just described. One of 5.5 mm. (no. 3960, cut coronally in 20-micron sections) proved to be a particularly reliable index because all the blood vessels of the head stand out very clearly, engorged with a remarkable natural injection (fig. 3). This embryo belongs to the same age group as no. 2841, and the general arterial configuration is similar, the basilar artery being in early formation in both. A dorsal fragment of each mandibular artery extends laterally from the carotid for a short distance, to be lost in capillaries just cranial and dorsal to the first pharyngeal pouch, as found in the embryos described above. The hyoid artery is definitely larger and much longer than the mandibular artery. It supplies the more dorsolateral part of the hyoid bar and terminates caudoventrally about midway between the ventral limits of the seventh and ninth nerve roots. Of particular significance is the fact that the ventral pharyngeal artery at its origin is large, at least four times the size of the mandibular artery. It divides into ventromesial and dorsolateral branches at the cranial level of the hyoid bar. The more prominent lateral branch, which continues the main stem, extends into the mandibular bar, giving off large branches to its substance before continuing cranially and dorsally over the pharynx to the mandibular nerve root (fig. 3*a*, *c*). This terminal branch is comparable to the "Oberkieferast" of the "external carotid" (ventral pharyngeal artery) described by Fuchs. In this embryo, therefore, it is obvious that the substance of the first pharyngeal bar is supplied not by the mandibular artery, but by the ventral pharyngeal artery, although the second bar continues to be partially supplied by the hyoid artery. This arrangement persists until the end of the "branchial" period, when the stapedial artery is formed.

Meanwhile, the most caudomesial part of the developing maxillary process, in contrast with the craniomesial part supplied by the primitive maxillary artery, may continue to be supplied by the small mandibular artery. This was noted particularly in a 6.8-mm. embryo which is more advanced than the above group (no. 6504, cut sagittally in sections of only 6 microns). Owing to unequal environmental growth, the hyoid artery has assumed a more caudal direction behind the first pharyngeal pouch (see below) and the mandibular artery a more cranial direction over the oral cavity (contrast right and left sides of the younger embryo, shown in fig. 3*b* and *c*). This apparent change in course is also seen in Tandler's models of the rat embryo. The first pouch is now relatively wider than before, and the mandibular

[6] It should be noted that in Congdon's illustration of a model of no. 1380 (his pl. 2, fig. 34), the artery labeled "later mandibular" and shown taking a dorsal course is actually a part of the primitive trigeminal artery to be described in a later section of this paper.

artery has obviously lost all connections of any significance with the lower jaw region. Its domain is the roof of the oral cavity in the area to be traversed by the future superficial petrosal nerve (compare figs. 30 and 31, pl. 3). The question arises whether the artery seen at this stage represents the original mandibular artery or a collateral maxillary branch, as reported in the rat by Tandler (the "infraorbital branch of the dorsal remnant of the first aortic arch"). Since, however, the interruption of the primitive mandibular artery certainly takes place in a position dorsal rather than ventral to the pharynx, and since in no embryo of this series could its remaining dorsal end be followed into the subpharyngeal region, it seems unnecessary to assume more than a slight forward shifting in the direction of the original stem of the artery. Whenever the mandibular artery could be identified at all, following its interruption, it was confined to the region dorsal and cranial to the oral cavity, that is, the developing maxillary process. In some cases during later stages, it constitutes a so-called Vidian artery, although an internal carotid branch accompanying the superficial petrosal nerve is apparently small and inconstant in human development (see below).

It is of interest to compare the next embryo of our series, no. 617, of 7 mm., with that of Tandler's human "stage III," of the same length and practically identical in general arterial development, including the third, fourth, so-called "fifth," and pulmonary aortic arches. The mandibular artery has the same configuration in both embryos. It is shown in a horizontal head section by Tandler in his text figure 27, with its more distal threadlike part labeled "first aortic arch in degeneration [*Rückbildung*]," and also in our figure 31, plate 3. In our horizontal reconstruction, the superficial petrosal (Vidian) nerve is illustrated and was traced to a position just caudal to the mandibular artery, which as yet retains its original caudolateral course. By contrasting figure 31 with the earlier stage shown in figure 30, plate 3, it will be seen that the internal carotid origin of the mandibular artery, as also that of the hyoid artery, appears to have shifted cranially in reference to the delimiting pharyngeal grooves and pouches. As a result, both the mandibular artery (or "maxillary" artery, as it could now be called at the expense of its being confused with the primitive maxillary artery arising from the more distal carotid; see also fig. 13*b, c, d*) and the superficial petrosal nerve which it will soon accompany, in some cases at least, are in position for descent into the roof of the oral cavity during the early "post-branchial" phase (fig. 32, pl. 3).

Both in our embryo and in Tandler's, the distal part of the hyoid artery passes into the caudal confines of the second or hyoid bar. From its more cranial point of origin on the internal carotid, it is now directed caudolaterally as in the corresponding stage in the rat. But it should be noted that the terminal area of the artery remains the same as in the earlier embryos, that is, it courses between the roots of nerves VII and IX, ventral to the tympanic nerve, and is lost near the ninth nerve root and second pharyngeal pouch. These apparent changes in origin, position, and direction of the hyoid artery are explained by the unequal growth in its environment between the 4- and 9-mm. stages. The growing predominance of the mandibular bar, with its advancing maxillary process, over the hyoid and glossopharyngeal bars, the attendant caudal curve of the seventh nerve, and the close grouping of the seventh to tenth nerve roots can best be visualized by a comparison of figure 30 with 31 in plate 3, and figure 2 with 4.

The hyoid artery has not yet given off a branch directed toward the territory of the first aortic arch, but the apparent forward shifting in its carotid origin makes conditions more favorable for such a formation. The ventral pharyngeal artery is conspicuous both in Tandler's embryo (labeled variously "external carotid" and "ventral aorta") and in our no. 617. Furthermore, in our embryo, it was observed to terminate in a plexus around the mandibular nerve root, after skirting the mandibular end of the developing chorda tympani nerve along its mesial border (fig. 31, pl. 3); this approximation of nerve and artery is also seen in the other embryos of this stage, notably no. 163, of 9 mm. (fig. 4*a*).

Tandler's next succeeding stage is a 12.5-mm. embryo which is comparable to our 16- to 18-mm. embryos in general arterial and cerebral development. Here, the stapes primordium and its artery, including all three branches, are well advanced. To determine the formation of these branches, including their main stem, a series of embryos between 9 and 14 mm. were studied and several were graphically reconstructed. Two of 12 mm. (no. 1771, fig. 5; no. 6520) and two

of 14 mm. (no. 940, fig. 6; no. 6258), which are all definitely younger than Tandler's 12.5-mm. embryo, show a large cranially directed offshoot from the hyoid artery. This collateral immediately passes through the condensation of mesenchyme representing the stapes and therefore constitutes the definitive stem of the stapedial artery. It lies dorsal to the first pharyngeal pouch just under the marked caudal curve in the course of the seventh nerve, and is in the same position as the anastomotic branch between the dorsal remnants of the first and second aortic arches, which appears in both rat and rabbit embryos. In one of the 14-mm. embryos just mentioned, it was possible to trace a definite anastomosis between the new stapedial collateral and what is undoubtedly the distal remnant of the ventral pharyngeal artery. This specimen (no. 6258, not available for reconstruction) is cut horizontally in 10-micron sections and the smaller arteries are marked by a good natural injection. Since it so closely resembles in body form and in general arterial and nervous development our reconstructed embryo of 14 mm. (no. 940), the distal connections of the stapedial artery in the former embryo could easily be superimposed freehand on the basic reconstruction of the latter (fig. 6; fig. 32, pl. 3). In this significant early stage of the postbranchial phase, the stem of the hyoid artery no longer appears to be within the hyoid bar, owing to the environmental shifting and unequal growth foreshadowed in the preceding stage. Rather, the origin of the hyoid artery from the internal carotid appears to be actually in front of the first pharyngeal pouch and cleft as shown in both horizontal (fig. 32, pl. 3) and bilateral (fig. 6) views of embryo no. 940. The collateral branch of the hyoid artery, which is now the stem of the stapedial artery, is therefore in an entirely favorable position for anastomosis with vessels in the lateral mandibular substance. In all the embryos of this stage, the distal end of the hyoid artery, so prominent in earlier stages, is reduced to a small remnant which passes almost directly caudally along the tympanic branch of the ninth nerve, an association observed with regularity in both younger and older embryos. This hyoid remnant, including its large proximal part now serving as the stem of the stapedial artery—in other words, the whole length of the original hyoid artery—is retained as the small caroticotympanic branch of the adult internal carotid. The ultimate dwindling or interruption of the stapedial artery during embryonic life occurs not at the carotid artery, but at the stapes, that is, at the most proximal end of the stapedial collateral given off by the hyoid artery (figs. 33–36, pl. 3). Therefore, the adult caroticotympanic artery represents, strictly speaking, not a remnant of the stapedial, as noted by various anatomists, but the whole embryonic hyoid artery.

The definitive stem of the stapedial artery, whose formation has just been described, gives off immediately beyond the stapes a cranial offshoot (the future supraorbital division) which can be traced a short distance lateral to the Gasserian ganglion. The continuation of the main artery (as seen in a 14-mm. embryo, no. 6258), which represents the lower or maxillomandibular division of the stapedial, courses ventrally along the mandibular nerve to join a plexiform vessel at the root of the chorda tympani nerve. This vessel may be followed caudally along the chorda tympani for about one-half its distance (i.e. the mandibular as contrasted with the hyoid part of the nerve); here it turns somewhat mesially in the direction of the component of the external carotid artery, where it dwindles and is lost (fig. 32, pl. 3). The primitive external carotid, representing the proximal end of the ventral pharyngeal artery (Congdon, 1922), now originates from the third aortic arch, which is about to lose its identity in becoming the definitive common carotid artery. The subpharyngeal vessel, with which the stem of the stapedial has made the anastomosis just described, undoubtedly represents the distal end of the earlier ventral pharyngeal artery, which, it will be recalled, extended to and accompanied the mandibular end of the chorda tympani nerve (fig. 31, pl. 3). The present interruption of the ventral pharyngeal artery has occurred as a result of the disintegration of the aortic arch system and the new suprapharyngeal connections now established by the stapedial artery between the realms of the first and second aortic arches. In summary, the stapedial artery forms as a collateral of the hyoid artery and annexes the distal end of the ventral pharyngeal artery to form the stem of its maxillomandibular division. This anastomosis occurs at the mandibular root of the fifth nerve, so that the mandibular is the first of the three definitive branches of the stapedial to be formed. The maxillary branch is a secondary derivative of the mandibular branch, and the supraorbital

division is a derivative of the main stem of the stapedial artery. The point of origin of these three stapedial branches from their common stem, it will be noted, is not the same as the configuration in the rabbit, which in turn is unlike that in the rat (see p. 231 above).

The fate of the primitive mandibular artery was indicated during the early postbranchial stages of this series. As shown above, the small remnant of this artery, arising from the internal carotid, lies in the path of the developing superficial petrosal nerve during the branchial period (fig. 31, pl. 3). Later, when the nerve has reached and passed closely by the lateral aspect of the internal carotid, the mandibular artery may sometimes be seen to accompany the nerve for a short distance. In one embryo (no. 544, 11.5 mm., an injected specimen) this artery was prominent enough on the right side to be followed cranioventrally for some distance into the well developed maxillary process (fig. 40, pl. 4). It could not be definitely determined whether it communicated with the laterally placed maxillary branch of the stapedial artery, then in formation, because of the rich venous plexus in this region; however, in an 18-mm. embryo (no. 1390) one could trace a questionable plexiform communication (fig. 7*a*). Failure to find a so-called Vidian artery in other embryos does not prove its nonexistence, but one can be certain that it does not at any stage in man attain the importance found in many adult reptiles or in the bat embryo, mentioned above. Several textbooks of anatomy describe an adult Vidian artery (or artery of the pterygoid canal) as a small inconstant branch of the internal carotid, arising within the petrosal bone just above the small caroticotympanic branch which represents the embryonic hyoid (stapedial) artery. This Vidian artery is sometimes said to anastomose with a branch of the descending palatine artery (from the internal maxillary). But more commonly, according to most anatomists, the artery in the Vidian canal is a branch of the distal (originally stapedial) part of the internal maxillary artery. The present observations on the history of the mandibular artery may be summarized as follows: Following the involution of the first aortic arch, the dorsal end of the succeeding mandibular artery is interrupted at the craniodorsal margin of the first pharyngeal pouch, and its abbreviated dorsal remnant, therefore, remains entirely suprapharyngeal, limiting its domain to the maxillary region; it sometimes constitutes a small Vidian artery after the superficial petrosal nerve develops as far as the contiguous part of the artery at its origin from the carotid; it does not enter into the formation of any branches of the stapedial artery, as is the case in the rat, and is ultimately taken over in many cases by the internal maxillary artery. Both the Vidian (embryonic mandibular) and the caroticotympanic (embryonic hyoid and stapedial) arteries are significant in the adult as potential sources of collateral circulation between the internal and external carotid arteries.

## THE OPHTHALMIC ARTERY

At the time when the maxillomandibular division of the stapedial artery is formed, as discussed in the foregoing account, one finds only the stem of origin for the future supraorbital division. This arises just beyond the stapes from the common stem supplying the bulk of the upper and lower jaws, and is first traced to the region of the eye in embryos of approximately 18 mm. Its history cannot be comprehensively discussed without a consideration of the development of the ophthalmic artery, since man, like many lower forms, shows a close association between these respective components of the adult external and internal carotid systems.

No description of early stages in the development of the eye vessels in man has appeared. Versari, in a series of papers appearing from 1900 to 1923 (references given in Mann, 1928), and Dedekind (1908–1909) dealt with older stages, beginning with embryos of approximately 20 mm. Mann, in a 1928 monograph on the development of the human eye, recapitulates the work up to that time and devotes considerable attention to fundamentals of optic vascularization, although unfortunately it is difficult to orient oneself in reference to the description and illustrations of her younger embryos. These authors were chiefly concerned with the complicated details of development and retrogression in the strictly ocular blood system, and do not give any clear idea of the related stages in the formation of the whole ophthalmic artery, including orbital as well as optic branches. Since the present study shows that the permanent adult stem of the ophthalmic artery does not appear

until the 18-mm. stage and many of its branches are not recognizable until later, it is important to know, in reference to all earlier specimens, from what part of what parent stem any "ophthalmic artery" under discussion arises and what are the environmental relations. The detailed study of the eye vessels in the rabbit by Fuchs (1905) appears to be the only complete account of the subject, though he also was not always specific as to the larger related arteries in successive stages. His work reveals that all the arteries to the region of the eye, orbital and ocular, have a relatively complex history which the present study showed to be equally involved in man.

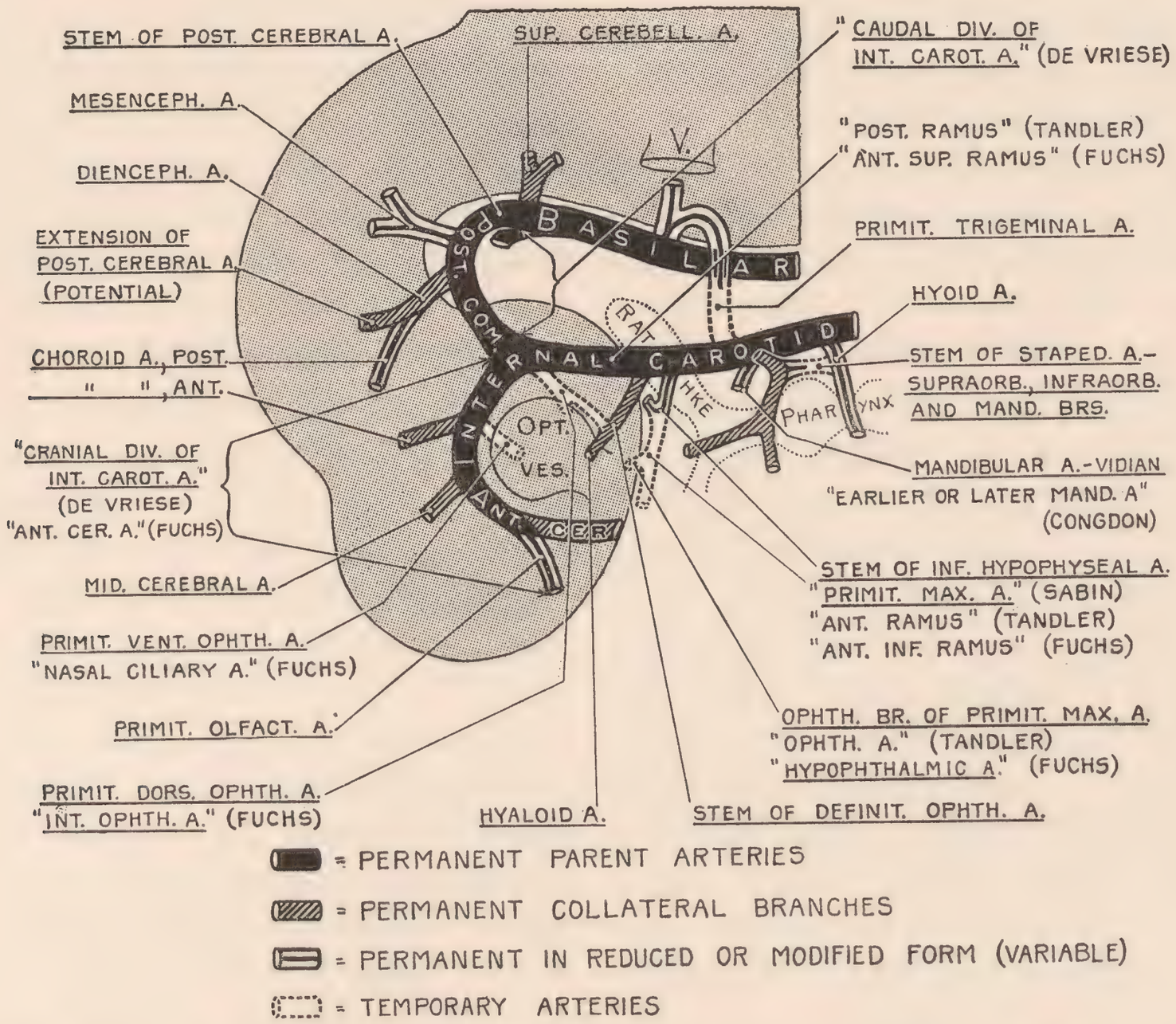


FIG. 12. Composite diagram of the arteries around the embryonic forebrain to illustrate differences in terminology employed by various writers. Their names are in parentheses, their designations are in quotation marks, and those adopted or orginated for this study are underlined. This variation in terminology must be noted in reference to any correlation of the development of the human stapedial and ophthalmic arteries with that described for other species.

A discussion of the earliest arterial supply to the region of the eye cannot be pursued without some clarification of terminology, since the literature shows a considerable diversity in both the naming and the interpretation of the many vessels of this territory. These differences together with the terms adopted in the present discussion have been illustrated in diagrammatic form in figure 12. For instance, Tandler (1902) gives several figures of a young rat embryo (his stage II) wherein the arteries cranial to the first aortic arch are named in a misleading if not erroneous manner. His illustrated model "stage II" (his pl. 3, fig. 4) shows the "cerebral carotid" (primitive internal carotid) dividing into two branches of equal size. The "anterior ramus," passing ventrally just in front of the oral cavity, gives off the ophthalmic artery (his text fig. 6). The "posterior ramus" extends cranially toward the brain. It is clear (see the transverse sections of the same embryo in his text figs. 4, 5) that the anterior ramus, just mentioned, in passing caudal to the optic vesicle cannot be in the position of the terminal cerebral part of the primitive internal carotid, which he also designated as the "anterior ramus" in his later stages (his text fig. 9). Again, "posterior ramus" as designated in the successive stages of his text figures 6, 9, and plate 3 has two meanings: in one sense it is used for the primitive internal carotid extending to the forebrain, and in the other it refers to the later caudal division of the

internal carotid (posterior communicating artery). Fuchs (1905) points out that Tandler's "anterior ramus" is identical with an artery which gives off a temporary "hypophthalmic" branch in the very young rabbit embryo, and which he calls the "anterior inferior ramus" in contradistinction to the terminal cerebral part of the carotid, named the "anterior superior ramus." The anterior and the anterior inferior rami of these writers not only are the same artery, but are clearly comparable with the first prominent branch of the primitive internal carotid consistently found in early human embryos and called in the present study the "primitive maxillary artery" (see below).

Fuchs also complicates the terminology by referring to the cranial division of the internal carotid, curving cranially around the optic stalk, as the "anterior cerebral artery," and does not always identify various collateral branches of this artery, although several are illustrated in certain figures. As fully clarified by De Vriese (1905), what Fuchs and others called the "anterior cerebral" is the primary cranial, as contrasted with the primary caudal, division of the primitive internal carotid. Only in later stages when the cranial division gives off the middle cerebral collateral branch, which defines the full cerebral extent of the adult internal carotid, does the anterior cerebral stem of origin become definitive. The whole terminal part of the internal carotid artery, which lies against the brain wall, together with several primitive derivatives (fig. 12), is concerned during its inception with optic supply, and its various landmarks must be kept well in mind in connection with the following descriptions.

## Development of the Ophthalmic Artery in the Rabbit

Since the present study does not pretend to cover all details of the subject, but has revealed considerable similarity between the history of the human ophthalmic artery and that of the rabbit, it seemed advisable to outline the latter as described so fully by Fuchs (1905). He found that during the 9th day in the rabbit embryo the supply to the optic vesicle comes from two branches of the primitive internal carotid artery. These are the "anterior superior ramus" (the terminal cerebral part of the internal carotid), extending craniodorsal to the vesicle, and the "anterior inferior ramus" (primitive maxillary artery), passing along its ventroposterior aspect or base (fig. 12). By the 10th day, the terminal end of the carotid has formed its cranial ("anterior cerebral") and caudal ("posterior communicating") divisions and the former supplies the most cranial aspect of the vesicle. The internal carotid, opposite its caudal division, gives off the "primordium of the permanent internal ophthalmic artery." It should be noted that the stem of the "internal ophthalmic" in the rabbit is comparable (in reference to its destination, though not its origin) to that of the ophthalmic artery of man and is so named in contradistinction to the "external ophthalmic artery" of the rabbit, which is developed later from the external carotid. In addition to the sources of optic supply just mentioned, there is also a branch from the anterior inferior ramus of the carotid (primitive maxillary artery); this is a highly transitory "hypophthalmic artery" (fig. 12). All these arteries soon lose themselves in a capillary net, the "primordium of the choriocapillaris," surrounding the vesicle. At the 11th day, the "hypophthalmic" branch has disappeared, now being represented by an elaborate plexus in the area traversed by the former vessel. The plexiform vessels of the "choriocapillaris" have met in a "ring artery"[7] at the periphery of the lens. At the end of the 11th and beginning of the 12th day, the numerous carotid branches to the "choriocapillaris" are reduced in number. The two most prominent are as follows: the "primordium of the long nasal ciliary artery," which comes from the cranial division ("anterior cerebral") of the internal carotid and supplies the more ventral or "nasal" half of the eye, and the previously established stem of the internal ophthalmic artery, which now has a "long temporal ciliary branch" supplying the more "temporal" or dorsal half of the eye; these arteries lose themselves in the choriocapillaris. From the caudal side of the "ring artery," a "blind bud" arises to pass mesially between the optic cup and lens into the outer part of the fetal ocular cleft[8] (choroid fissure); this is the "primordium of

[7] Mann (1928) notes that Versari called the counterpart of this vessel a vein in the human embryo and that, in view of the "peculiarities of both fetal and adult ocular circulation," it is better to call it simply the "annular vessel."

[8] Mann argues against the use of the term "choroid fissure," since it "tends to create the impression that the cleft has some connection with the choroid." Furthermore, the origin of one of the primitive ophthalmic arteries of the human embryo bears a definite relation to the anterior choroidal artery, which supplies the cerebral choroid plexus. The term she suggests, "fetal ocular cleft," will be used here.

the primary hyaloid artery." At the end of the 12th day, the internal ophthalmic artery has two divisions, the stem of the long temporal ciliary branch and the "primordium of the definitive stem of the hyaloid artery." The primary hyaloid artery, which was previously formed, has become dilated into the "bulbus arteriosus hyaloideus," which is now connected with the ring artery by four branches, two dorsal and two ventral. The mesial end of the "bulbus" soon develops a secondary anastomosis with the choriocapillaris through the medial aspect of the fetal ocular cleft. By the 13th day, the internal ophthalmic is the largest optic branch of the internal carotid, and its derivative, the definitive stem of the hyaloid artery, has anastomosed with the "primordium of the primary hyaloid artery" lying within the fetal ocular cleft, thereby completing the definitive hyaloid artery. The bulbus is then resolved into the branches of the vascular tunic of the lens. The remaining branch to the eye is a prominent long nasal ciliary artery whose origin, exactly as in the earlier stages, is from the most cranial curvature of the cranial division of the internal carotid (Fuchs' "anterior cerebral artery").

The 14th day in the rabbit marks the significant second phase of related development, in which the stapedial artery has formed, including a "bulbo-orbital" branch associated with the frontal nerve from the ophthalmic division of the fifth nerve. The internal ophthalmic artery now makes an anastomosis with the long nasal ciliary artery, thus annexing this primitive branch of the most distal end of the internal carotid. Fuchs emphasizes that at this stage the internal ophthalmic artery, including bilateral long ciliary and hyaloid branches, constitutes the sole supply to the eye, while the stapedial artery, through its orbital branch, alone supplies the orbit. From the 15th day, there take place the final changes which characterize the optic and orbital arteries in many vertebrates. In the rabbit these are, in brief, the annexation by the external carotid of practically all the arteries to the region of the eye. The branches of the stapedial artery are first taken over by the external carotid as in the human embryo. Later, the orbital branch of the external carotid (formerly stapedial) not only develops all the orbital arteries (such as branches to the lacrimal and Harderian glands), but also takes over, through its external ophthalmic division, both the long nasal and temporal ciliary arteries, previously established as branches of the internal ophthalmic artery. Except for a variable anastomotic connection, this annexation leaves to the small internal ophthalmic branch of the internal carotid only the role of supply to the retina through the proximal remnant of the hyaloid artery. As will be described below, the pattern of development in the human embryo up to the 20-mm. stage closely parallels that of the rabbit up to, but not including, the final stages. The last stages of human development, in contrast with that of the rabbit, are characterized by the complete predominance of the internal over the external carotid artery in the supply to both eye and orbit.

## The Primitive Ophthalmic Arteries in Man

In the 1912 review by Evans it is noted, in statements based on earlier reports, that the ophthalmic artery is the first branch of the internal carotid to develop and that in embryos of 7 mm. it divides into bilateral long ciliary and hyaloid branches. This is illustrative of Sabin's (1917) warning against a premature naming of embryonic vessels. The ophthalmic arteries of this stage are only part of an elaborate plexus, components of which will later give rise to but a small part of the adult ophthalmic artery. However, a large primitive branch, which is temporarily directed toward and gives off tributaries to the base of the optic vesicle, does represent the first cerebral branch of the internal carotid artery. This ventrally directed branch was first seen in embryos of 3 to 4 mm. and was noted by Tandler (see above) and Sabin in reference to rat and pig embryos respectively. Fuchs showed that supply to the optic vesicle by this artery, through a temporary "hypophthalmic" branch, is exceedingly transitory in the rabbit, and the present study shows this finding to be equally true for the human embryo. Since the stem of the vessel persists in relatively reduced form to supply the most mesial parts of the maxillary region around Rathke's pouch, Sabin's term "primitive maxillary artery" (1917, not discussed by her but so designated in the labeling of a 6.5-mm. pig), is used in the present account.

Although supply to the optic region by this primitive maxillary artery is lost at an early stage, its sub-

sequent history is a matter of interest. The artery is prominent in all the earliest embryos of this series and, although not named, is either described or illustrated in connection with those of Ingalls (1907), Elze (1907), and Davis (1923). Like the mandibular artery described above, this vessel is mesial rather than lateral and takes no part in the formation of the maxillomandibular division of the stapedial artery. Its origin is well ahead of the first aortic arch, from the most mesial curve of the internal carotid, and its course is primarily ventral through the tissue bordering the neck of Rathke's pouch. In two embryos of 4 mm. (no. 836, fig. 38, pl. 4; no. 588, fig. 1 and fig. 44, pl. 5) the artery vascularizes the base of the undifferentiated optic vesicle and the neighboring caudomesial tip of the prosencephalon. In two older embryos of between 5 and 6 mm. (nos. 1380 and 3960), wherein the optic cup and stalk are in beginning formation, the artery is still relatively large and conspicuous, with its primary termination at the prosencephalon. It gives off both a mesially directed branch, passing between the base of Rathke's pouch and the brain wall (fig. 3*a*), and a lateral branch to the developing cleft in the optic vesicle (left side of embryo, fig. 3*b*) which is most suggestive of the transitory "hypophthalmic artery" seen in the 10-day rabbit embryo (fig. 12). After the 5- or 6-mm. stage, when its primitive role of supply to the cerebral and optic regions is transferred to branches of the more cranial part of the internal carotid, the primitive maxillary artery dwindles in size (fig. 2). Between the 6- and 12-mm. stages it was not always identified on both sides and, when seen, could usually be traced for only a short distance ventrally from the carotid. As seen in the lateral views of successive stages up to 14 mm., the artery is consistently at the level of the maxillary branch of the fifth nerve and maintains its original position just lateral to the base of Rathke's pouch.

In an 18-mm. embryo (no. 1390, fig. 7), in which the neck of the hypophyseal diverticulum has become much attenuated, there is a small branch of the internal carotid (two branches on one side) in the position of the primitive maxillary artery. These now pass more mesially than ventrally toward the small craniopharyngeal canal, to which the wide neck of Rathke's pouch is now reduced, and border the caudal part of the hypophyseal diverticulum (two branches were seen on one side in one earlier embryo, no. 2841 of 5.3 mm.). In a 24-mm. embryo (no. 632, injected by Evans) the lumen of the craniopharyngeal canal is obliterated, and the anterior hypophysis is fairly well differentiated and partly surrounds the infundibular process. The internal carotid, proximal to the ophthalmic artery, gives off two bilaterally symmetrical branches which could be followed mesially only as far as the hypophyseal border, although they undoubtedly enter this structure (fig. 8*a;* fig. 35, pl. 3). The most cranial branch, arising just below the definitive stem of the ophthalmic artery, apparently anastomoses with the caudal branch skirting the caudal margin of the hypophysis,[9] after giving off a branch passing between the gland and the brain. One or both of these carotid branches must represent the primitive maxillary artery, which now appears to be concerned with vascularization of the hypophysis.

As is now known, the blood supply of the adult hypophysis, which includes a portal system, is very complex, and the material of this paper adds no significant developmental details. Several points of general interest, however, should be noted. In the first place, it is clear that the primitive maxillary artery, in spite of its early association with Rathke's pouch, does not become an artery of supply to the anterior hypophysis, which is derived from this pouch. The emergence of the permanent stem of the ophthalmic artery during the 18-mm. stage, in a position cranial to the primitive maxillary artery, tends to confine the origin of the latter to the intracavernous part of the internal carotid. It has been shown that the anterior and posterior hypophyseal lobes, in a series of mammals including the rhesus monkey and man (Wislocki and King, 1936), have independent arterial supplies derived from two separate sources. That to the anterior hypophysis (pars distalis) and pars tuberalis arises from numerous minute "pial" branches from the intracranial internal carotid (that is, above the stem of the ophthalmic artery) and the posterior communicating arteries. The less highly vascularized infundibular process is supplied by one or two bilateral "dural" branches of the intracavernous carotid, that is, the carotid segment which gave origin to the primitive maxillary artery. The "superior hypophyseal arteries" (Wislocki, 1937) to the anterior hypophysis in

[9] It should be remembered that the caudal or posterior border of the hypophysis in the embryo head, which is bent forward over the chest, represents the ventral or inferior border in the adult.

the human embryo were first identified in the present series in an injected embryo of 11.5 mm. (fig. 40, pl. 4). They are represented at this stage by numerous plexiform branches from the carotid and posterior communicating arteries; these branches spread mesiocaudally over the diencephalic floor and surround the more cranial borders of Rathke's pouch. In this embryo the neck of the diverticulum is still wide open, the infundibulum is undeveloped, and the primitive maxillary artery, as in other embryos of this stage, is only a short carotid twig. As noted above, no derivative of the primitive maxillary artery was definitely traced to the developing hypophysis until the 20- to 24-mm. stage (fig. 35, pl. 3), and the vessels seen at this time must undoubtedly represent the inferior hypophyseal arteries. This finding is in line with Wislocki's statement that the "infundibular process does not receive its definitive vessels until it attaches to the dura" and thus is "vascularized at a relatively late period and very slowly."

A further detail reported in the adult dog by Dandy and Goetsch (1911) is of interest in connection with the fate of the primitive maxillary artery. Here the supply to a small epithelial body lying at the base of the gland in the floor of the sella, namely, the "parahypophysis which is evidently a remnant of Rathke's pouch," comes bilaterally from two small arteries of cavernous carotid derivation. The more cranial branch passes directly mesially from the carotid to the parahypophysis. The caudal one also sends a branch to the parahypophysis before joining its fellow of the opposite side to form the sole artery of the posterior hypophysis. Certainly one, and perhaps both, of these arteries must represent the primitive maxillary artery which is first seen in 3- to 4-mm. human embryos as a prominent carotid branch, bordering the lateral margins of the hypophyseal diverticulum. After the early and complete loss of its primary role of supply to the prosencephalon and base of the optic vesicle, the stem of the primitive maxillary artery is retained to become incorporated ultimately in the formation of the inferior hypophyseal arteries.

The configuration of all the primitive arteries to the developing human eye during various stages will now be described. In the youngest embryo of this series (no. 2053, 20 somites) the primitive carotid terminates in a plexus at the caudal aspect of the emerging optic vesicle (fig. 37, pl. 4). In a 4-mm. embryo (no. 836) the internal carotid extends farther cranially to surmount the dorsal aspect of the optic vesicle, giving off en route the well defined primitive maxillary artery (described above), which proceeds ventrally to the base of the prosencephalon (fig. 38, pl. 4). In another 4-mm. embryo (no. 588, fig. 1) the terminal cerebral part of the carotid has branched into its cranial and caudal divisions. In place of the diffuse plexiform supply of the earlier stages, there are several more definite branches to the optic vesicle; the most notable one, which has been named for this account the "primitive dorsal ophthalmic" artery, leaves the carotid at its point of division. In a comparable embryo, that of Ingalls (1907, pictured by Evans, 1912, fig. 417), this was prematurely labeled the "ophthalmic artery."

During the 4- to 6-mm. stages, the optic vesicle differentiates into the short optic stalk and the optic cup, with a wide-mouthed indentation which is beginning to enclose the lens primordium. Beginning with this period, six stages in the development of the ophthalmic arteries are illustrated in semidiagrammatic form in figure 13. As is shown in detail in figure 3*b*, the two sides of the embryo (no. 3960) on which the composite figure 13*a* is based show two successive stages in arterial development around the eye. On the left side the optic stalk is short and, owing partly to an asymmetrical rotation of the forebrain, is close to the origin of the primitive maxillary artery, which still gives a branch to the ventrocaudal aspect of the cup where the fetal ocular cleft is forming. But on the right side, all direct connection between this artery and the eye has been lost. On this side there has formed a large, more distal branch of the internal carotid, arising at the point of its cranial and caudal division. This artery immediately subdivides; its shorter branch, passing to the mesial face of the optic vesicle, is the primitive dorsal ophthalmic artery first seen in less advanced embryos (figs. 1, 2); the recent and more prominent derivative is an artery which pursues a long lateral course to pass over the caudodorsal rim of the optic cup. This somewhat plexiform vessel encircles the caudoventral aspect of the ectodermal lens pit and then dips into the depression which represents the fetal ocular cleft. It is obviously the counterpart of the primitive internal ophthalmic artery which supplies the ring artery of the lens in the 11-day rabbit embryo (see figs. 191–194 of Fuchs,

1905). Mann (1928, figs. 31, 158) pictures a model of a 5-mm. human embryo which is comparable to our 5.5-mm. embryo (fig. 3). Although it is difficult to orient oneself as to the surrounding structures in her illustrations, it is highly probable that the vessels shown, one of which, "the future hyaloid artery," is "penetrating the interior of the cup, the other vascularizing its margin," belong to the prominent dorsal ophthalmic branch of the carotid just described. Although the artery does appear to come from "below" in her illustrations because of the angle at which the model of the eye is viewed, the carotid origin of the vessel would be dorsal to the eye in reference to the whole embryo head. It should be borne in mind that the primitive dorsal ophthalmic artery is actually caudodorsal in relation to the cup and stalk, just as an opposing primitive artery, to be described presently, is cranioventral in this relation. Like the 11-day rabbit embryo, this specimen shows several other irregular branches from the cranial division of the internal carotid artery as it curves cranial to the stalk; these supply the more cranial and ventral regions of the optic vesicle.

The next available stage is represented by embryo no. 163 of 9 mm. (fig. 4*a*), which has a good natural injection of vessels in the region of the eye. Here the primitive dorsal ophthalmic artery, which arises in the position of the permanent internal ophthalmic artery of the rabbit and was seen in the preceding human stage, is again large and conspicuous. However, after coursing directly laterally it now appears to end abruptly in a manifold plexus over the dorsal aspect of the optic cup, and no definite single branch can be traced into the cup or fetal ocular cleft as formerly. The plexus covering the optic cup is connected, however, with a more or less complete annular vessel which surrounds the periphery of the lens at the outer margin of the cup; this vessel in turn communicates with a sinus-like blood vessel within the fetal ocular cleft (fig. 13*b*). The latter vessel (a primitive hyaloid artery) also communicates through plexiform channels with the more mesial part of the optic capillary mesh and drains into the multiform veins in the maxillary process. Detailed stages, analogous to the complex development of the hyaloid artery described in the rabbit, were not determined for the present study, but sections of no. 163 (9 mm.)[10] are similar in microscopic appearance to those of the 12-day rabbit embryos illustrated by Fuchs (his

[10] For this embryo there exist only tracings without orientation guide lines, and, since the optic vascularization was seen to be highly plexiform, no attempt was made to reconstruct it in detail.

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FIG. 13. Six stages in the development of the ophthalmic artery and the related supraorbital division of the stapedial artery. (Ventral views showing the right side of the embryo.)

*a*. Approximately 4 to 5 mm. The primitive maxillary artery sends a temporary branch to the base of the optic vesicle, but the primordial cup and lens are supplied by a large primitive dorsal ophthalmic artery.

*b*. Approximately 9 mm. The primitive dorsal ophthalmic artery now terminates in a plexus investing the optic cup, which is also supplied by a new ventral ophthalmic artery. An annular vessel surrounds the lens at the margin of the cup and connects with an irregular primitive hyaloid vessel within the fetal ocular cleft. Note the relative position of the hyoid and ventral pharyngeal arteries in comparison with those of the preceding figure.

*c*. Approximately 14 mm. Both dorsal and ventral ophthalmic arteries are elongated and the dorsal one has developed a direct branch to the lens, the definitive hyaloid artery. A collateral (stapedial) branch of the hyoid artery has captured the distal end of the ventral pharyngeal artery, the proximal end of which is now represented by the stem of the external carotid.

*d*. Approximately 18 mm. The elongated stem of the primitive dorsal ophthalmic artery has been replaced by a more direct vessel, the permanent stem of the ophthalmic artery. A secondary anastomosis joins its hyaloid branch to the primitive ventral ophthalmic artery, which is subsequently interrupted mesially beneath the cartilage primordium of the orbital roof. The stapedial artery has formed its two primary divisions, and the supraorbital one passes into the orbital cavity and borders the ophthalmic nerve.

*e*. Approximately 20 mm. The supraorbital division of the stapedial artery, the stem of which is dwindling, has developed several orbital branches and is being annexed by the ophthalmic artery, whose ocular branches were established in the preceding stage. Note the arterial ring by means of which the ophthalmic artery passes around to the dorsal side of the optic nerve. Following a similar formation, the internal maxillary artery reaches the outer side of the mandibular nerve root.

*f*. Approximately 40 mm. The conformation of the ophthalmic artery is recognizably adult following the interruption of two vessels, namely, the cranioventral segment of the arterial ring around the optic nerve; and the supraorbital division of the stapedial artery, whose orbital part has been augmented to form the lacrimal artery. The remainder of the supraorbital division is incorporated in the anterior branch of the middle meningeal artery.

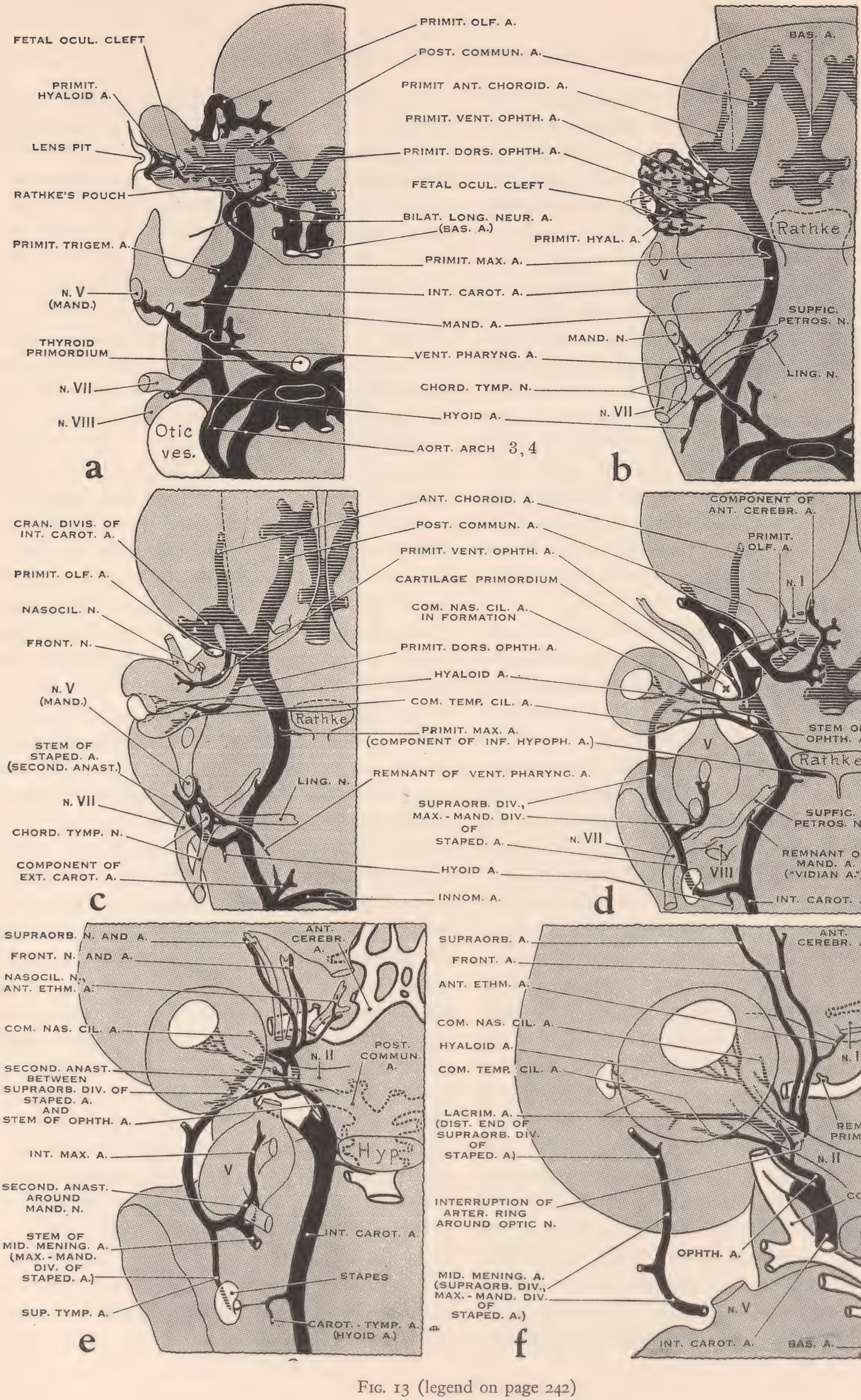


FIG. 13 (legend on page 242)

figs. 20, 21). It is obvious that the irregular blood vessel in the fetal cleft, probably comparable to the primitive "bulbus hyaloideus" of the rabbit and called at this stage in man the "hyaloid artery" by earlier writers, is not yet directly supplied by any one branch of the internal carotid. In other words, any connection between a hyaloid artery of this stage and the only conspicuous ophthalmic artery now present (the primitive dorsal artery) is very indirect. This fact was noted by Elze (1907) in reference to his 7-mm. embryo and discussed more fully by Barniville (1914) in one of 8.5 mm. There appears to be no evidence against the assumption that the complex process of development of the hyaloid artery reported in the rabbit is largely repeated in the human embryo. Certainly it is not until well along in the succeeding stage (11 to 14 mm.) that the primitive hyaloid artery establishes direct connection with the previously established primitive dorsal ophthalmic artery through the mesial end of the fetal ocular cleft.

In looking for an explanation of the circuitous development and late appearance of the definitive hyaloid artery, one must remember that the primordial ectodermal lens, which is relatively far removed laterally from the only available source of blood, namely, the internal carotid, gradually becomes overhung by the developing rim of the optic cup. Furthermore, the fetal ocular cleft developing in the more ventral part of the optic vesicle soon becomes diametrically opposed, in reference to the developing stalk and bulb, to the nearest source of blood, which is the dorsally placed internal carotid near its cranial and carotid divisions (compare figs. 2 to 6). As soon as the capillary mesh (primitive choriocapillaris) begins to envelop the optic cup (according to Mann, this occurs "pari passû with the development of pigment in the outer layer of the cup" between the 7- and 10-mm. stages), this plexus also is interposed in the path of the previously established primary ophthalmic artery (primitive dorsal ophthalmic), which formerly extended without plexiform interruption from the internal carotid artery to the lens. It is to be expected, therefore, that the detour made to the lens by the primitive dorsal ophthalmic artery, passing over the caudodorsal margin of the cup into the lateral (outer) end of the developing fetal ocular cleft (fig. 3), will eventually be replaced by a more direct route through the mesial end of the cleft (fig. 5). It will be seen that, even after establishment of the hyaloid artery as a direct branch of the primitive dorsal ophthalmic artery, the route taken by the hyaloid branch, as well as by its parent artery already supplying the dorsal aspect of the eye, remains circuitous. With further development of brain, optic stalk, and eye, it becomes more so. In the human embryo, as contrasted with the rabbit, a second short cut from the internal carotid to the lens and bulb is eventually established by the appearance of the permanent stem of the ophthalmic artery during the 14- to 18-mm. stages (fig. 7). These later changes will be described in more detail presently and are shown in semidiagrammatic form in figure 13.

As noted above, the most conspicuous ophthalmic branch of the internal carotid artery, in embryos of 7 to 12 mm., is the primitive dorsal artery supplying the caudodorsal parts of the vascular network around the optic cup. In these embryos, the cranial division of the internal carotid gives off, opposite the origin of the anterior choroidal artery, another less obvious but definite optic branch (fig. 4*a*); this vessel, arising from the most cranial and ventral segment of the internal carotid, was named for this study the "primitive ventral ophthalmic" artery. Like the "long nasal ciliary" branch of the "anterior cerebral" in an analogous stage of the rabbit (figs. 17, 19, 23 of Fuchs, labeled *r.o.* and *c.l.n.*), it supplies the cranioventral and more mesial part of the plexus investing the cup. Early in this study, interest was aroused as to the identity and significance of this artery in its later form. For instance, it is much more conspicuous in embryos of 16 to 24 mm., particularly those sectioned sagittally (figs. 7, 8), and by this time is a small, dwindling, though strikingly constant branch of considerable length which courses ventrally, at least, as far as the base of the optic stalk. Fuchs' findings in the rabbit offered a clue to its identity. Its origin was traced back to a 5.5-mm. embryo (no. 3960, described above) in which the arterial supply to the primitive stalk, cup, and lens could be identified with a considerable degree of certainty. It was seen again in more advanced form in three embryos of a later age group (nos. 617, 163, 1121, from 7 to 11.8 mm.). Since the cranial division of the internal carotid at this stage curves closely around the short

optic stalk, the primitive ventral ophthalmic artery is also short and passes directly caudally for only a slight distance to reach the plexus on the mesial face of the optic cup (fig. 4*a*). In following through successive stages, it is clear that with the growth of the forebrain in one direction and the elongation and ventral shifting of the optic stalk and cup in the other, the origin of this artery and the area originally supplied by it become considerably withdrawn from each other. These changes are best understood by comparing figures 4 to 7 (see also fig. 13).

By the time the 18-mm. stage is reached, the primitive ventral ophthalmic artery has shifted to a course which is more ventral than caudal and has become considerably elongated in its course to the mesial and cranial aspect of the optic cup. In embryo no. 1390 of 18 mm. (fig. 7; fig. 33, pl. 3) the permanent stem of the ophthalmic artery has just appeared and clearly shows a common temporal ciliary branch to the caudodorsal aspect of the optic cup. The future common nasal ciliary branch of the ophthalmic artery cannot be definitely traced. The primitive ventral ophthalmic branch of the carotid, however, courses to the anterior margin of the optic stalk, which it accompanies beneath the cartilage primordium that is now beginning to separate the cranial and orbital cavities. At this stage there probably exists within the orbit at least a plexiform anastomosis between the stem of the ophthalmic and the primitive ventral ophthalmic artery, but the close association of ophthalmic veins in this region made positive identification impossible. In the next stage (seen in four embryos of 21 to 24 mm., figs. 8, 9) this elongated and dwindling primitive branch of the carotid is still present on both sides and appears to terminate definitely at the margin of cartilage around the optic nerve. Furthermore, a common nasal ciliary branch of the ophthalmic artery has now appeared. It is in the same position as the distal end of the primitive ventral ophthalmic artery, which has obviously just been annexed by the stem of the ophthalmic artery (insert, fig. 33, pl. 3). This anastomosis, taking place ventral to the optic nerve, is usually with the hyaloid branch of the ophthalmic stem (see below) and is followed by interruption of the primitive ophthalmic branch of the carotid just proximal to the secondary anastomosis. The remaining carotid remnant of the primitive ventral ophthalmic artery, so constantly seen at the 20-mm. stage, was not identified in two older embryos of about 40 mm. It is probable, however, that remnants of both the primitive ventral and dorsal ophthalmic arteries are frequently retained in the adult as minute branches to the region of the optic chiasm. Detailed illustrations of the arteries from the anterior circle of Willis in several mammalian forms (Hofmann, 1900; Wislocki and King, 1936) and in the human adult (see drawing of the arteries of the hypophyseal region in a pregnant woman, fig. 6 of Wislocki and King) suggest this assumption. Similar branches were also noted by the writer in a 5-month fetal brain injected by Professor Max Brödel.

It is not until the 12- to 14-mm. stage that an internal carotid branch entering the mesial end of the fetal ocular cleft may be identified for the first time. This definitive hyaloid artery branches from the elongated primitive dorsal ophthalmic artery, which now courses caudoventrally before reaching the dorsal aspect of the eye and is recognizable as the common temporal ciliary artery (fig. 5). Although both hyaloid and temporal ciliary arteries are present, it is important to note that the carotid origin of their parent stem is not that of the unequivocal adult stem of the ophthalmic artery, which first appears in the succeeding stage; instead, it still arises slightly caudal or proximal to the origin of the posterior communicating artery (fig. 5; figs. 40, 41, pl. 4). It is apparent that the primitive dorsal ophthalmic artery, which at an early stage entered the outer or lateral end of the fetal ocular depression after coursing over the dorsal margin of the optic cup, and was next observed, during the 7- to 12-mm. stages, to supply this region only through an intermediate capillary plexus on the cup, is the same artery which now sends a direct branch to the mesial end of the fetal ocular cleft. However, details of the secondary anastomoses and differentiation (comparable with those described for the rabbit by Fuchs) which bring about this result were not observed. This temporary ophthalmic artery, which is still primitive both in origin and in course, now resembles the permanent stem of the internal ophthalmic of the rabbit at an analogous stage (Fuchs' fig. 27). As was described above, Fuchs showed that the adult stem of the internal ophthalmic artery in the rabbit is represented early and first supplies the

choriocapillaris and ring artery, and thus indirectly the lens. Later, coincidently with its acquisition of hyaloid and temporal ciliary branches, the destination and course of the same artery are modified in keeping with the growth changes of brain, optic stalk, and eye. Three stages of this process were diagramed in his text figure 2 (p. 106). This illustration makes it clear that the stem of the internal ophthalmic retains its primitive carotid origin opposite the posterior communicating artery, which originally allowed a direct lateral approach to the dorsal part of the optic cup, and that it is, therefore, gradually forced by environmental changes to pursue a lengthening caudoventral as well as lateral course before it may develop the secondary anastomosis which annexes its hyaloid branch. Although the original stem of the ophthalmic artery is retained in the rabbit, it will be seen that in the human embryo it is replaced by a secondary artery which permits a more direct route to the area it supplies.

The stem of the ophthalmic artery at its adult point of origin was first identified in embryos of approximately 18 mm. Note in figure 7 that it arises from the internal carotid, lateral to Rathke's pouch, in a position which is caudoventral to the optic nerve and therefore far removed from the junction of the carotid with the posterior communicating artery. Both hyaloid and common temporal ciliary branches are present as in the preceding stage. It is obvious that this stem represents a caudal "migration" along the carotid of the primitive dorsal ophthalmic artery seen in the preceding stages. In embryo no. 1390 (18 mm.) and, more obviously, in one of the same stage (no. 144 of 16 mm.), there are dwindling carotid remnants in the position of the primitive dorsal ophthalmic artery, which position is, of course, well distal to that of the permanent ophthalmic stem (fig. 7). In no. 390, of 15.5 mm. (like no. 1390, in age group xix; injected by Brödel), the new stem of the ophthalmic artery, near its origin, could be seen to communicate with two of these distally placed and somewhat plexiform carotid branches which spread toward the root of the optic nerve. On one side of a younger embryo of 12.5 mm. (fig. 5) the primitive dorsal ophthalmic leaves the carotid very close to the posterior communicating artery, but it is more proximally placed on the other side. In another embryo of 11.5 mm. (fig. 40, pl. 4), which is somewhat more advanced in general, the artery on both sides is even farther removed from the junction of the posterior communicating artery and, therefore, closer to its adult origin. But in both these embryos the course of this primitive stem of the ophthalmic artery is devious. Since its origin is still nearer to that of the posterior communicating artery than to the base of the optic stalk, the artery is forced to proceed caudally for some distance before swinging ventrolaterally to reach the fetal ocular cleft. In three other embryos of 12 to 14 mm. (nos. 940, 511, and 899) the ophthalmic arteries are still plexiform and primitive in that no one vessel can be traced to the fetal ocular cleft on either side. Apparently the early postbranchial period is one of varying adjustment concerning arterial supply to the eye. As referred to in connection with the lengthening and dwindling of the primitive ventral ophthalmic artery (common nasal ciliary artery of the rabbit), the growth changes in brain and eye force a considerable separation of the carotid origin of the primitive ophthalmic arteries from the territory that they supply. Comparing the illustrations of human embryos with those of the rabbit (in which the internal ophthalmic artery of the adult retains its primitive origin opposite the posterior communicating artery; see Fuchs' fig. 27, 29), one sees that the interval of separation becomes relatively much greater in the human form. Because of the far greater expansion of the cerebral hemispheres in man, which draws the arteries radiating from the circle of Willis dorsally and finally caudally, there is a gradual elimination of the unfavorable primitive origin of the ophthalmic artery. It appears to be gradually "pulled along" the internal carotid in a movement similar to the migration of the dorsal segmental and visceral branches along the aorta. In the case of the ophthalmic artery there is evidence that the vessel moves along the parent artery by means of a series of anastomotic loops (a process called anastomotic progression by Streeter, 1918, in reference to other vessels), and that its ultimate stem represents a secondary branch of the carotid which has annexed the branches of the primitive dorsal ophthalmic artery. Since the adult ophthalmic artery arises from the part of the carotid which is caudal to the optic nerve, it can most directly supply the fetal ocular cleft in the caudoventral aspect of the nerve.

## Relation of the Ophthalmic Artery to the Supraorbital Division of the Stapedial Artery

Various stages leading to the formation of the permanent adult stem of the ophthalmic artery, including its optic branches (the hyaloid, temporal, and nasal common ciliary), have just been described. No orbital branches of the ophthalmic artery have appeared at the 16- to 18-mm. stage. But meanwhile, the supraorbital division of the stapedial artery has emerged; it was traced to the orbit for the first time in an embryo of 18 mm. (fig. 7). It is known from the work of Tandler (cited by Evans, 1912) that this artery becomes a part of the middle meningeal artery, whose stem of origin is represented by the common stem of the maxillomandibular division of the stapedial artery. It has also been noted in most textbooks of anatomy that a branch of variable size from the anterior division of the adult middle meningeal may either anastomose with the ophthalmic artery or, as a definite anomaly, give origin to the lacrimal and other orbital arteries. The embryonic developments behind such variations in man have not been described.

In an 18-mm. embryo (no. 1390), the supraorbital vessel could be traced to the area where the ophthalmic branch of the fifth nerve divides into supraorbital (or frontal) and nasociliary branches; more specifically, it appears to terminate between the two nerves in the area just cranioventral to the optic nerve (fig. 33, pl. 3). As described above in reference to this embryo (p. 245), the distal part of the primitive ventral ophthalmic branch of the carotid, which also passes to the cranioventral side of the optic nerve, is about to be annexed by the ophthalmic artery as its common nasal ciliary branch; this takes place through an anastomosis ventral to the optic nerve. The common temporal ciliary branch of the ophthalmic stem has been established at an earlier stage. The fact that the temporal ciliary and the hyaloid arteries are the primary branches of the ophthalmic stem, and the nasal ciliary a secondary branch, may well be confusing in view of Keibel's statement in chapter 16, page 238 of the Keibel and Mall *Manual of Human Embryology* (1912) that "the nasal a. ciliaris communis gives off the a. hyaloidea [though] in many cases the a. hyaloidea arises at the point of division of the a. ophthalmica." The history of formation and the final configuration of the three optic branches are, however, entirely compatible if one visualizes the embryonic topographic relations. The common temporal ciliary (primitive dorsal ophthalmic) artery, related to the caudodorsal border of the optic nerve and eye, gives off the hyaloid artery. The latter branch, penetrating the optic nerve, is, of course, situated between the temporal ciliary and the primitive ventral ophthalmic artery coming from the internal carotid to the opposing anteroventral aspect of the nerve and eye. Therefore, the subsequent anastomosis, whereby the ophthalmic artery annexes its nasal ciliary branch ventral to the nerve, would commonly be between this branch (distal end of the primitive ventral ophthalmic artery) and the nearest branch of the ophthalmic stem, namely the hyaloid artery. After the primitive ventral ophthalmic branch of the carotid is interrupted just proximal to this anastomosis, the hyaloid artery does appear to arise from the resulting common nasal ciliary branch of the ophthalmic artery (see insert, fig. 33, pl. 3).

In embryos of approximately 20 mm., the supraorbital branch of the stapedial artery forms an anastomosis with the ophthalmic artery (fig. 8). In no. 6202 (21 mm.; fig. 34, pl. 3) this new connection, dorsal to the optic nerve, is between the ophthalmic artery, just proximal to its three optic branches, and the branch of the supraorbital division which now accompanies the length of the nasociliary nerve. Before this stage, the ophthalmic artery supplied only the eye, the surrounding orbital territory being supplied by the stapedial artery as shown in figure 34, plate 3. In a generally more advanced embryo of 24 mm. (no. 632, injected by Evans) the configuration of the orbital arteries is more primitive and plexiform than in the 21-mm. specimen, and demonstrates an intermediate stage (fig. 35, pl. 3). Here, the optic nerve, at the origin of the three primary optic branches of the ophthalmic artery, is surrounded by an arterial ring, the components of which represent several secondary anastomoses. The less well defined ventral portion represents in part the anastomosis between the primitive ventral ophthalmic branch of the carotid and the ophthalmic stem—the anastomosis which forms the common nasal ciliary artery (described above). The dorsal part of the ring is larger and probably of more recent origin. It is the channel

whereby the future definitive ophthalmic artery is permitted to pass, as typically in the adult, from the ventral to the dorsal aspect of the optic nerve. As will be seen in the next stage, a small part of the more ventral portion of this arterial ring is normally destined to disappear; all other parts are retained in the final configuration. In this embryo one sees three orbital branches of the supraorbital division of the stapedial artery, which arise near the craniomesial aspect of the optic nerve, each accompanying a division of the ophthalmic nerve. They are the frontal, supraorbital, and anterior ethmoid arteries.

An embryo of 39 mm. (no. 6203, enlarged 25 times and therefore permitting considerable detail in the graphic reconstructions) was interesting because the right and left sides illustrate two further successive stages in the development of the ophthalmic artery (fig. 36, pl. 3). The supraorbital division of the former stapedial artery, whose stem is dwindling following annexation of its branches by the internal maxillary artery, is now represented by the middle meningeal artery coursing lateral to the Gasserian ganglion. Beyond the orbit the meningeal artery is prolonged in the dural covering of the brain by a collateral branch of the supraorbital division. The original orbital segment of the supraorbital division, just distal to this collateral, is now interrupted at the orbital margin (compare figs. 35 and 36, pl. 3). In brief, the more primitive connection between the stapedial (now middle meningeal) and ophthalmic arteries has now become obliterated. In addition to its frontal and supraorbital branches, formed at the last stage, the ophthalmic artery has been essentially completed by the acquisition of its lacrimal branch, which terminates at the primordium of the gland. When the somewhat more primitive arteries on the right side of this embryo shown in figure 36, plate 3, are compared with the preceding figure 35 (24 mm.), the origin of the lacrimal artery can be observed. It is obvious that at least its proximal part represents the strictly orbital segment of the supraorbital division of the stapedial artery, which first entered the orbit at the 18-mm. stage. In a common form of adult variation, the anterior branch of the middle meningeal artery gives off an anastomotic branch which enters the outer end of the sphenoid fissure (or a separate foramen), and joins the lacrimal artery. This retention of an embryonic channel is readily understood on comparing the configuration of the 20- to 40-mm. stages (pl. 3).

The less mature development of the ophthalmic arteries on the right side of the 39-mm. embryo also shows a retention of the arterial ring which formed around the optic nerve at the 20-mm. stage. The cranioventral part of the ring represents the secondary channel whereby the permanent stem of the ophthalmic artery annexed the primitive ventral ophthalmic artery to form its common nasal ciliary branch. The dorsal part is the still later secondary channel which joined the supraorbital division of the stapedial artery to the ophthalmic artery. As seen in figure 36, plate 3, the cranioventral segment of this arterial ring is interrupted on the more advanced left side of this embryo, resulting in a typically adult configuration. It is of interest to recall here that the adult ophthalmic artery, after entering the orbital cavity underneath the optic nerve, may keep to this ventral position (15 per cent of the cases noted by Wolff, 1933) instead of passing immediately up over the lateral and dorsal aspect of the nerve to give off all, or nearly all, of its orbital branches as typically. This variation obviously results from the interruption of a dorsal rather than ventral segment of the arterial ring around the embryonic optic nerve.

Finally, the embryo in figure 36, plate 3 gives the explanation for variations reported in the origin of the common nasal ciliary artery of the adult. This small vessel (or vessels) has been frequently noted as leaving the main stem of the ophthalmic artery after the latter has passed around to the dorsal side of the optic nerve. Such an origin is already seen on the left side of this embryo (no. 6203). On the other hand, Quain (1844, cited by Wolff) says that the nasal ciliary and central retinal arteries usually arise in common below the nerve. As may be seen in comparing the two sides in our embryo, either one origin or the other would be determined by the point at which the more ventral segment of the arterial ring around the optic nerve is interrupted. The central retinal (embryonic hyaloid) artery of the adult is the only branch which is invariably given off before the ophthalmic passes from the ventral to the dorsal aspect of the optic nerve. This small artery tends to be anchored by its early penetration into the fetal ocular cleft, which is always represented in the ventral surface of the nerve. In connection with the his-

tory of the common ciliary arteries and the site of the fetal ocular cleft, it should be remembered that there is both an apparent and a real rotation of the optic stalk and eye. The first is merely the result of the lifting of the embryo head off the chest so that the fetal ocular cleft, always in caudoventral position, is invariably represented in the more inferior aspect of the adult optic nerve (Wolff, 1933). The second is the actual change in direction of the optic nerves from their original lateral plane to the adult anterolateral plane, resulting in a gradually diminishing angle between the optic axes. It is then realized that the arteries originally supplying the opposing caudodorsal (also more lateral) and cranioventral (more mesial) aspects of the optic cup are brought around to the temporal (lateral) and nasal (medial) surfaces of the eye. These changes must be kept in mind in the identification of the developing common ciliary arteries of the embryo, which are important from the standpoint of orientation in early stages.

## THE PRIMITIVE TRIGEMINAL ARTERY

Evans (1912), in describing the origin of the primitive internal carotid artery from the end of the first aortic arch, called attention to certain smaller, irregular "presegmental branches of the dorsal aorta," which he identified in three early human embryos (6 to 15 somites), noting that remnants were apparently present in the Ingalls 4.9-mm. embryo (1907). He spoke of these branches as "very transitory and attracting our interest chiefly because they represent the first vascular sprouts sent out by the dorsal aorta, which, directed towards the sides of the medullary tube, are directly responsible for the formation of the vena capitis medialis." Sabin (1917) established the fact that the so-called "vena capitis medialis" serves only temporarily as a vein for the head, and renamed this channel, which is closely applied to the hindbrain wall, the "primitive rhombencephalic vessel." She emphasized that it is essentially a neural vessel which disappears entirely in the development of an extensive capillary plexus out of which ultimately both arteries and veins arise. From this plexus are developed what she named the "bilateral longitudinal neural arteries" (precursors of the basilar artery), which "form as an anastomosis between all the neural arteries from the dorsal aorta extending from the carotid arteries at the base of the optic stalk to the tip of the spinal cord." Streeter (1918) also described this "primordial hindbrain channel" (primitive rhombencephalic vessel) as arising in association with "irregularly placed slender communications from the dorsal aorta in the hindbrain region." The present study has revealed one of these branches, henceforth to be referred to as the "primitive trigeminal artery," to be relatively constant and often of considerable prominence in the early human embryo.

Evans (1912, footnote p. 622) made brief reference to the "presegmental branches of the dorsal aorta" as reported in two vertebrates. Tandler (1902) found a row of them in the rat at the 3-mm. stage. In his figure 8, five branches are shown arising at regular intervals from the paired ("dorsal") aorta opposite the interrupted first aortic arch back to the primitive hypoglossal artery opposite the fourth arch. At the 5-mm. stage, according to his figure 14, the longitudinal neural artery ("cerebral vertebral") is apparently more or less continuous cranially with the posterior communicating artery and caudally with the hypoglossal artery. Remnants of three presegmental branches remain. The one opposite the dorsal remnant of the first aortic arch (mandibular artery) in the trigeminal region is still uninterrupted, thereby forming an accessory link between the internal carotid and the longitudinal neural arteries. De Vriese (1905) found two relatively constant vessels in the rabbit embryo in front of the temporary hypoglossal artery, one at the level of the otic vesicle, the other near the Gasserian ganglion. She mentioned the possibility of their being the homologues or vestiges of "cephalic segmental arteries" related to the fifth, eighth, and twelfth nerves. At the 4- to 5-mm. stage (her pl. 15, fig. 2; pl. 16, fig. 2), the caudal divisions of the internal carotid (future posterior communicating arteries), reinforced by the primitive "trigeminal" and "auditory" (otic) arteries and connected with cranial extensions of the primitive "hypoglossal" and first cervical segmental arteries, have formed parallel tracts in the hindbrain region. De Vriese called these vessels the "primitive spinal arteries," which, of course, are synonymous with both the "cephalic" or "cerebral vertebrals" of early writers (for instance, Evans, 1912) and the "longitudinal neurals" of Sabin (1917). As the basilar artery is formed, through the

consolidation of the bilateral neural arteries, the trigeminal, otic, and hypoglossal anastomoses dwindle (De Vriese, pl. 17, figs. 21–24; pl. 15, fig. 12). In the 7-mm. embryo shown in her plate 16, figure 29, only the trigeminal branch remains uninterrupted. Fuchs (1905) in his study of the eye vessels of the rabbit embryo also showed what is undoubtedly the same trigeminal "Verbindungsast" at an early stage (see illustrations of a model, his figs. 7, 8). Sabin (1917) noted that her plate 6 of a 29-somite chick "shows none of the primitive arteries which connect the primitive rhombencephalic vessel directly with the dorsal aorta," but that in the 7-mm. pig (her pl. 7) "it is very interesting that two of these original arteries still persist and take part in the formation of this new longitudinal artery." A branch, in the trigeminal region, connecting the first aortic arch with the primordial hindbrain channel is apparently also shown in Evans' injected 5.7-mm. pig (1912, his fig. 394). Congdon (1922) stated, "there can be little doubt, in the light of . . . observations . . . on the development of the longitudinal neural arteries in other forms, that all the cranial members of the segmental series, as far back as the first cervical, contribute by anastomosis to the formation of the longitudinal neural arteries" in the human embryo. In spite of such references, partly buried in the important studies just mentioned, no description of these vessels in the human embryo has appeared. According to our material, the small presegmental branch or branches in the otic region, and also the hypoglossal artery, are exceedingly transitory. But the role played by the trigeminal branch in the early stages of development of the neural and basilar arteries is a considerable one, and its late persistence may take a prominent form. In the course of this study the primitive trigeminal artery was first observed in two embryos of 11 and 14 mm., in which it constitutes a large anastomotic branch between the carotid and basilar arteries. For the purpose of tracing its development in an orderly manner, however, the following description begins with the earliest embryo in which it could be identified, and ends with its final disappearance.

The cephalic end of an embryo of 20 somites (no. 2053 of 3 mm., age group xi) was reconstructed graphically in right and left lateral and coronal views. Davis (1923), who modeled and described the whole embryo, noted that the first pair of aortic arches is complete and the second in formation. There are two branches springing from the first arch. The first branch, which represents the primitive internal carotid, runs cephalad and "communicates with deep and superficial head plexuses in the region of the optic vesicle. The second branch leaves the arch just lateral to the first and, passing dorsally, ends in similar plexuses adjacent to the trigeminal ganglion." This second branch is the earliest representation of what is here called the primitive trigeminal artery. As can be seen in figure 37, plate 4, and figure 43, plate 5, this bilateral dorsal branch arches just mesial to the trigeminal ganglion and communicates with the primordial hindbrain channel ("primitive rhombencephalic vessel" of Sabin). The primordial channel is also supplied by two or three slender, irregularly placed vessels in the otic region. An initial stage in the formation of the second part of the primary head vein is represented by a channel which is present on the left side only, in a position superficial to the eighth nerve, and is a derivative of the primordial hindbrain channel. This specimen is illustrative of the first stage of vascular development described by Streeter (1918), during which the primordial vascular system of the head is established.

This 20-somite embryo may be compared with one of 24 somites (age group xii) reported by Johnson (1917), which shows a branch almost certainly identical with the primitive trigeminal artery just described. In his illustration it originates from the first aortic arch just caudal to the primitive carotid and is labeled *a.cer.?*. Johnson writes that it "extends dorsally and medially and terminates in the region of the posterior end of the prosencephalon, a short distance behind the anterior end of the notochord. I am unable from the specimen or from other descriptions to identify this vessel with any degree of certainty, but presume that it gives rise to one of the cerebral arteries." Comparison of Johnson's figure 1 (showing the arteries) and figure 2 (showing the veins) in plate 5 indicates that this branch of the first aortic arch is undoubtedly connected with the primordial hindbrain channel as in our comparable embryo.[11]

[11] In accordance with the interpretation of writers preceding Sabin's paper in 1917, Johnson refers to all parts of the primordial hindbrain channel, which temporarily drains the brain at this stage, as "veins of the anterior cardinal system" (see his p. 102).

A second stage in the development of this trigeminal artery is seen in a 4-mm. embryo (no. 836; figs. 38, 39, pl. 4), in which the third aortic arch has just become complete, and which illustrates the first stage in the differentiation of supply and drainage channels of the head described by Streeter. One may now distinguish fragments of the paired longitudinal neural arteries being formed in the region of the future basilar artery. At the level of the Gasserian ganglion, opposite the mandibular artery, which has succeeded the first aortic arch, there is the primitive trigeminal artery identified in the preceding stage. It must be noted, however, that it now arises from the primitive internal carotid and terminates in a fragment of the longitudinal neural artery, and that these vessels represent, respectively, the paired aorta and the primordial hindbrain channel of the earlier 20-somite embryo. The trigeminal artery is somewhat plexiform on the right but is an unequivocal channel on the left. The primordial hindbrain channel has disappeared and the primary head vein has formed, showing a prominent ventral tributary (later the stem of the inferior maxillary vein). This vein courses just lateral to the arterial anastomosis between the internal carotid and longitudinal neural arteries in the trigeminal region, and represents the more superficial parts of the head plexus seen in the 20-somite embryo.

At the eighth nerve and otocyst, opposite the second aortic arch, there are two or three other bilateral and more slender communications between the paired neural arteries and dorsal aorta; these were also definitely noted in the 20-somite embryo (fig. 37, pl. 4). The bilateral neural arteries are in early formation on the ventral hindbrain wall, being more prominent in the otic and trigeminal regions, and difficult to trace in the areas beyond and between (figs. 38, 39, pl. 4). Caudally the neural artery is lost in a capillary bed at the level of the ninth nerve. The internal carotid at this stage terminates in a plexiform channel dorsal to the optic vesicle, and has not yet formed its cranial and caudal divisions. At the mesencephalon, there are plexiform fragments of the caudal division (the future posterior communicating artery), which later becomes the most cranial source of blood to the neural artery.

A later stage in the development of these arteries is well illustrated by a more advanced embryo of 4 mm. (no. 588; fig. 1). Here the fourth aortic arch is complete, the pulmonary arch is in formation, and the first two arches have been succeeded by the irregular, plexiform mandibular and hyoid arteries. The primitive carotid, just ahead of the mandibular artery, bifurcates into two branches of equal caliber. One is the cerebral prolongation of the carotid artery, extending mesiocranially to the prosencephalon. The other is the primitive trigeminal branch, which arches laterally, dorsally, and then mesially to join the longitudinal neural artery (fig. 44, pl. 5). The neural arteries, which later consolidate to form the basilar artery, are more advanced than in embryo no. 836. They border a mid-line nonvascular strip on the ventral hindbrain wall and are everywhere connected laterally with the plexus enveloping the hindbrain; their extent is without interruption from the trigeminal region to the first cervical segmental arteries. The internal carotid has formed its primary cranial and caudal divisions, but the caudal one (future posterior communicating artery) dwindles into a capillary plexus at the mesencephalon, and no definite connection between it and the neural artery can be traced on either side. The chief supply to the neurals, as this specimen makes clear, is through the trigeminal arteries. On the right side of the embryo the trigeminal branch, in relative size, direction, and regularity of contour, actually appears to be the direct continuation of the carotid artery. One small connection between the carotid and neural arteries at the otocyst remains on the left side, and the primitive hypoglossal artery is interrupted on the right. In early stages, therefore, the bilateral longitudinal neural artery is supplied largely by the trigeminal artery; this supply is reinforced caudally by other more transitory presegmental branches of the paired (dorsal) aorta (or internal carotid) and also by the first cervical segmental artery.

Embryo no. 1380 of 5.5 mm., which is similar in general cranial development to that of 4.9 mm. described by Ingalls and also to our embryo no. 2841 (fig. 2), shows a somewhat later stage in the history of the primitive trigeminal artery. The definitive posterior communicating artery has just been formed by the union of the caudal division of the carotid (which it represents in greater part) with the bilateral longitudinal neural artery; however, this secondary anastomosis at the mesencephalon is relatively small and irregular. The trigeminal artery, though smaller on

the left side than on the right, still appears to be the chief cranial source of blood supply to the hindbrain, since the neural artery into which it empties is at least twice as large on each side between the level of the Gasserian ganglion and the otocyst than cranially at the midbrain. There also persists a small bilateral anastomosis between the internal carotid and neural arteries just ahead of the eighth nerve on one side and farther back at the otocyst on the other; these primitive otic arteries were not seen at any succeeding stage in this series.

In two embryos of 5 and 9 mm., which are comparable to no. 2841 of 5.3 mm. (fig. 2) in that the basilar artery is being formed, the trigeminal artery is present in prominent form. Reference should now be made to figure 3 (no. 3960, 5.5 mm., age group xiv). Here the trigeminal artery, as in no. 2841, constitutes a caroticoneural anastomosis, but the carotid end is cranial to the basilar end. Furthermore, the fact that the carotid end is smaller than the basilar on the left, and is plexiform at the basilar on the right, suggests an imminent interruption at this junction. In contrast, both posterior communicating arteries are large and regular and join one of the largest areas of coalescence of the two neural arteries. The caudal termination of each neural artery at the first cervical segmental artery is small and plexiform. In summary, the neural arteries, in this embryo, are supplied primarily by the posterior communicating division of the carotid but are still being reinforced by the primitive trigeminal branches in a manner reminiscent of a stage in the rabbit embryo described by De Vriese.

Another naturally well injected embryo (no. 4782 of 9 mm., of the succeeding age group xv, which is less advanced than no. 163 of 9 mm., fig. 4*a*) shows another variation of the trigeminal artery, as well as a somewhat later stage in the formation of the basilar artery. This specimen has not been photographed, but, since it was sectioned coronally, it was feasible to draw a freehand millimeter reconstruction of the ventral view as an aid in study of the slides. The basilar artery is nearly complete from the posterior communicating artery to the posterior level of the fifth nerve and Rathke's pouch, containing in this cranial segment only two small islands, each at the point where a bilateral set of branches is given off; the caudal part is still plexiform. On the left side, the advanced cranial end of the basilar appears to be directly continuous with the trigeminal artery, which arches laterally and is then directed mesially to join the carotid. This outer curve of the channel and the level of its position, between Rathke's pouch and the fifth nerve root, is exactly the configuration in all the embryos just described (pl. 5). No trigeminal artery (caroticobasilar anastomosis) is present on the other side of this embryo, and it is probably significant that here the posterior communicating artery is twice as large as on the side where the anastomosis persists. In other words, the internal carotid supplies the basilar on the right side through its permanent posterior communicating branch, but on the left largely through its temporary trigeminal branch.

In two reconstructed embryos of the early postbranchial period (no. 544, 11.5 mm., and no. 940, 14 mm., age group xvii), the basilar artery is fully formed, although irregular and retaining an island at its caudal end. In each, the vertebral artery is also complete, albeit very irregular, and the common carotid has just become definitive owing to the obliteration of the paired aorta between the third and fourth aortic arches. In view of this relatively late stage in general arterial development, and as compared with other embryos of this stage, all the cerebral arteries of these two specimens must be considered unusually primitive (figs. 40–42, pl. 4). Both embryos have retained the trigeminal or caroticobasilar anastomosis in striking form. On the right in no. 544 (fig. 41), the anastomosis is actually between the carotid and vertebral arteries through a long vessel paralleling the basilar artery. This lateral vessel is a late retention of the channel, prominent in many younger embryos (figs. 3–6), which originally supplied the more lateral and caudal regions of the hindbrain. An uninterrupted remnant of this primitive lateral basilovertebral anastomosis is seen again in no. 940 on the right side (fig. 42). Here the trigeminal artery connects the carotid with both the basilar and vertebral arteries by means of this lateral channel.

Another point of interest illustrated in the base views of these embryos (pl. 4) is the small size of the posterior communicating artery at its terminal part, which joins the basilar artery; this is seen on the left side of the embryo in figure 41 and on both sides in figure 42. Furthermore, the somewhat primitive vertebral artery in both embryos is also small in the upper part, which constitutes a segment of the first cervical

segmental artery (fig. 40). In these cases it is clear that the basilar artery is still being largely supplied by the prominent primitive trigeminal branch of the carotid. Only on the right side of no. 544 (fig. 41), where the primitive trigeminal anastomosis between the carotid and basilar arteries is interrupted, is the posterior communicating artery characteristically large at its basilar end. These embryos in general show a relatively late persistence of the stage wherein the basilar, representing the bilateral neural arteries, receives most of its blood from the trigeminal branch of the internal carotid.

There are several factors initiating the development and retrogression of this temporary embryonic artery. Dr. Streeter, early in this study, suggested that its formation is related to the precosity of the fifth nerve ganglion, and it was found to be related to the mesial border of this nerve at all stages (see the six figures of pl. 5). The eighth nerve and otocyst also attract presegmental branches from the paired aorta to the primordial hindbrain channel, as seen in the 20-somite embryo of 3 mm. These small otic branches, however, unlike the trigeminal, are seen only in the 3- to 4-mm. stages during the development of the second aortic arch, opposite which they form (figs. 37, 38, pl. 4). As soon as the second aortic arch dwindles, the primitive hypoglossal and first cervical segmental arteries, which accompany the corresponding nerves, supply the caudal end of the neural arteries (fig. 1). The primary branches of the first aortic arch, namely the internal carotid and trigeminal arteries, which had the head start, so to speak, at the 3-mm. stage, are now firmly established. They arose to supply the areas then undergoing marked differentiation, the primitive carotid branch taking care of the forebrain and optic vesicles, the trigeminal artery going to the most cranial part of the hindbrain. The prominent trigeminal artery and the smaller, more transient otic and hypoglossal arteries are all certainly related to the development of the brain in the region of the corresponding cerebral nerves (and developing ear), as suggested by De Vriese.

As has been shown above, the trigeminal artery is consistently found during the period preceding the caudal extension of the primary caudal division of the internal carotid to the mesencephalon, coincident with the development of this region and the emergence of the third and fourth nerves. At this time a secondary anastomosis joins the caudal carotid division with a cranial extension of the longitudinal neural artery to form the definitive posterior communicating artery, which usurps the role of the trigeminal artery as a temporary communicating artery between the carotid and the arteries of the hindbrain. The completed posterior communicating artery lies in the direct path of the blood flow from the internal carotid, in contrast with the trigeminal artery, which by this time leaves the carotid at an angle approaching 90°. Asymmetrical rotation of the forebrain, which is often observed in embryos of this stage, may relatively widen the interval between the carotid and the base of the hindbrain or alter the relation between the origin and destination of the trigeminal artery and, therefore, be another factor in its eventual interruption. In one embryo (no. 1121 of 11.8 mm.) showing interrupted remnants of the anastomosis, the carotid end is well in front of the basilar, although they appear to be in line in the coronal view (fig. 45, pl. 5). In another embryo (no. 3960; fig. 3), described above, the carotid and basilar ends of the long unbroken but dwindling anastomosis are in the same relative positions as the remnants just mentioned. In other words, the trigeminal artery, in the stages immediately preceding its interruption, leaves the carotid at an acute angle which is the opposite of the original configuration in that its apex is pointed cranially instead of caudally; the artery, therefore, finally proceeds in the reverse direction to that of the blood flow in the parent carotid. In contrast, the two largest embryos of this series (12 and 14 mm.; pl. 4) which retain the anastomosis show the trigeminal artery leaving the carotid in a craniodorsal direction, as originally seen in the 4-mm. embryo (fig. 2).

Later on, the developing basal sphenoid cartilage is interposed in the area between the internal carotid and basilar ends of the primitive trigeminal artery. In the two embryos of 12 and 14 mm., which showed the temporary anastomosis in most conspicuous form, the consolidation of the mesenchyme in this region is just getting under way. Somewhat equivocal remnants of the anastomosis exist in several other embryos from 11 to 16 mm., but no definite uninterrupted channel was found beyond 14 mm. In a 23-mm. embryo (no. 966; fig. 47, pl. 5), where the basal cartilage is well advanced, there is a continuous but plexiform remnant on the left side; on the right, the dural fold,

extending dorsolaterally from the sphenoid, marks the terminal point of a basilar remnant. Again, in a 43-mm. embryo (no. 886; fig. 48, pl. 5), basilar remnants extend to the fifth nerve roots and ganglia only as far as the developing fold of dura.

In conclusion, attention is called to the striking adult anomaly which may result from a retention of the trigeminal or caroticobasilar anastomosis, as reported by more than a dozen writers. Quain (1844) pictured the anomaly in his atlas, and here the large anastomotic branch perforates the sphenoid bone. In De Vriese' case (1905; a 9-month fetus), as in that of Smith (1909) and others, the branch perforated the dura at the level of the posterior clinoid process. The cases in which the related basal arteries were described or illustrated (Quain; De Vriese; Hasenjäger, 1937) show a configuration comparable to that of the 11- to 14-mm. embryos just described. They are indicative of the embryonic stages when essentially all the hindbrain arteries are supplied by the trigeminal branch of the internal carotid system. For instance, the basilar artery, caudal to the anastomosis, and one or both vertebrals were relatively small, as were also the posterior communicating artery at its connection with the basilar on the side of the anastomosis. In Hasenjäger's case both posterior communicating arteries were absent, and there were other variations related to the anterior cerebral and vertebral arteries. This anomaly and others, pertaining to the arteries radiating from the circle of Willis, are noted and their embryonic derivations briefly discussed by the writer in a chapter of Dandy's 1944 monograph on intracranial arterial aneurysms (see composite diagram, his fig. 28).

## SUMMARY

An analysis of important stages in the development of the cranial arteries in the human embryo, based on graphic reconstructions of 22 specimens of the Carnegie Collection, is presented. The most significant changes in arterial development take place earlier than the critical stages in the formation of the dural veins as described by Streeter (1918), and plate 1, showing eight successive steps in the adaptation of the arteries to cranial growth changes from 4 mm. to birth, may be compared with Streeter's well known figure (his pl. 1), after which it was patterned.

The embryonic stages which were chosen to illustrate the formation of the head arteries are correlated with the transformation of the aortic arches described by Congdon (1922), and the whole is summarized in table 1. As noted in the table, the internal carotid, the basilar, and the vertebral arteries are formed during the first three stages, respectively, which belong to Congdon's "branchial" period (approximately 4 to 12 mm.). The fourth stage (12 to 14 mm.) is a significant transitional period in reference to both the aortic arches and the cranial arteries. The last three "postbranchial" stages (16 to 40 mm.) show the emergence of all the adult arteries, or their permanent adult stems of origin, and the completion of the circle of Willis. The various steps in the development of the cranial arteries can be followed by comparing the correlated illustrations of embryos in both lateral and horizontal views which were graphically reconstructed for text figures 1 to 11 and modified or simplified for plates 1 and 2.

Like vascular development elsewhere, that of the head arteries is characterized by a gradual dwindling or elimination of certain vessels, originally prominent, which serve temporary needs, paralleled by the elaboration of others. The emergence of the internal carotid artery during the involution of the first two aortic arches (fig. 1); the consolidation of bilateral neural arteries of the hindbrain to form the basilar artery (figs. 2, 3); and the secondary anastomoses between the upper seven cervical segmental branches of the paired aorta, giving rise to the vertebral artery (figs. 4, 5), were described more or less fully by Congdon (1922). The present study has concentrated on the formation of the various carotid and basilar branches. For instance, the history of the anterior cerebral artery is of particular interest. The primitive internal carotid of the 4- to 5-mm. stage has two major divisions arising near the summit of the optic vesicle: the caudal division, which later constitutes the posterior communicating artery after anastomosis with the bilateral neural arteries which precede the basilar artery; and the cranial division, which curves cranioventrally around the base of the optic vesicle (fig. 1). Although this latter division has often been called the "anterior cerebral artery," only a small part of it is

represented in the adult vessel. The cranial division of the carotid at its terminal end constitutes for some time a primitive olfactory artery supplying first the olfactory area (figs. 1, 2) and later the nasal fossa (fig. 4) and cavity (fig. 7). From this primary division of the carotid several collateral branches arise during later stages; these are, first, the anterior choroidal, secondly the middle cerebral, and finally the major part of the anterior cerebral artery. During the early postbranchial phase (12 to 14 mm.), the large primitive olfactory arteries are drawn mesially toward each other as the nasal fossae become approximated at the mid-line. Each has developed a craniomesial collateral supplying the emerging olfactory nerve root (fig. 32, pl. 3). By 18 mm., the collateral branch, which is a component of the anterior cerebral artery, begins to overshadow the primary artery (fig. 7). During the 20-mm. stage, when the anterior cerebral extends up between the cerebral hemispheres, the primitive olfactory artery sends an offshoot to the anterior perforated substance (figs. 8, 10) and is frequently retained in the adult as a variable striate branch of the anterior cerebral artery. The more constant medial striate (Abbie, 1937) or recurrent artery of Heubner, which arises from the anterior cerebral at the level of the anterior communicating artery, is very conspicuous in the embryo after 20 mm. (figs. 8–10). It dates far back in phylogenetic history and, together with the variable remnant of the primitive olfactory artery, represents early embryonic stages when a component of the future anterior cerebral artery is limited to the olfactory region.

The posterior communicating artery, which represents the original caudal division of the primitive internal carotid, remains relatively large throughout all the stages in this series. Until the completion of the vertebral artery in the early postbranchial phase, it is the channel through which the internal carotid supplies all the arteries of the hindbrain. As seen in plate 1, it primarily extends caudocranially from carotid to basilar arteries and gradually swings through a 180° arc as a result of the growing predominance of the cerebral hemispheres over the rest of the brain.

The definitive posterior cerebral artery cannot be identified unequivocally in this series of embryos, but it must be noted that its adult stem of origin represents the most distal (caudal) end of the original posterior communicating artery, and is, therefore, represented very early (figs. 2–4). Its ultimate extension emerges by means of an elaboration of one of the large diencephalic or mesencephalic branches of the posterior communicating artery seen in the 7- to 40-mm. embryos (figs. 16–21, pl. 1). These arteries are, of course, small branches of the posterior cerebral artery in the adult, and, as is the case with the formation of the anterior cerebral artery, a collateral branch finally overshadows the primary artery.

Of the cerebellar arteries, only the superior cerebellar becomes definitive during this series, appearing in the late branchial phase (7 to 10 mm.), when it supplies the metencephalon (fig. 4). However, the stems for both the inferior cerebellar arteries, anterior and posterior, often become recognizable among the numerous plexiform arteries which arise early to supply the region of the otic capsule and the myelencephalon (figs. 16–18, pl. 1). Not until a stage later than the present series, when the cerebellum surrounds the medulla, will the definitive destination of these arteries be established. In embryos between 20 and 40 mm., however, both arteries are represented by vessels which terminate in the large choroid plexus of the fourth ventricle (figs. 19, 20, pl. 1) which these arteries supply via small branches in the adult (fig. 11). Before the 40-mm. stage these arteries can usually be identified only tentatively as those which are larger and longer than numerous other associated basilar and vertebral branches which arise to supply the hindbrain regions, lying between the seventh and twelfth nerve roots massed in this area. Since these transverse branches are so often connected by longitudinal remnants of a prominent lateral channel (the primitive lateral basilovertebral anastomosis), paralleling the basilar artery and the cerebral part of the primitive vertebral artery, the arteries of the embryonic medulla long present a somewhat plexiform appearance (figs. 3–8). The variable origin in the adult of the anterior and posterior inferior cerebellar arteries, in contrast with the relative constancy of the superior cerebellar (Stopford, 1916), is thus readily explained (pls. 1, 2).

The external carotid system emerges in the early postbranchial period. As noted by Congdon (1922), the proximal end of the ventral pharyngeal artery, which succeeds the ventral parts of the first two aortic arches, is incorporated in the stem of the external

carotid artery (figs. 30–32, pl. 3). Its appearance coincides with the dwindling of the segment of paired aorta between the third and fourth aortic arches, which permits the third arch to become recognizable as the definitive common carotid artery (figs. 5, 6). The subsequent appearance of the various external carotid branches is illustrated in figures 7 to 11 (see also pl. 1) and discussed further in connection with the stapedial artery.

In the preparation of the outline covering the cranial arteries in general, three individual arteries merited special attention. Two of these, the temporary stapedial and the permanent ophthalmic, are interrelated and involved in a relatively prolonged series of modifications. Early stages in the formation of the stapedial artery, which originally pierces the stapes primordium and develops branches to the mandibular, maxillary, and supraorbital regions, have not heretofore been described in reference to the human embryo. As in many other vertebrates, the hyoid artery, which replaces the second aortic arch, is the carotid stem of the stapedial artery in man (figs. 1–4). Only the small root part of the hyoid artery, however, becomes incorporated in the stapedial artery, the greater part of the stapedial stem being a secondary cranial collateral of the hyoid vessel, which, by the late branchial phase (7 to 10 mm.), has assumed a relatively caudal course in the hyoid bar (fig. 4). By the early postbranchial phase, the proximal or carotid end of the hyoid artery appears to have been shifted from the caudal to the cranial level of the hyoid bar, where it gives off the stapedial collateral which passes craniоventrally into the mandibular bar to the mandibular nerve root, at the emergence of the chorda tympani nerve (figs. 5, 6; fig. 32, pl. 3). This phenomenon of arterial supply to the first pharyngeal bar by a vascular derivative of the second can be understood only by visualization of the unequal growth factors in the environment. The mandibular bar with its advancing maxillary process appears as though it pushed caudally the hyoid and glossopharyngeal bars, the components of which become closely approximated coincidently with the loss of definition between these originally well demarcated regions. These changes are illustrated in figures 30 to 32, plate 3, and also in plate 1, based on text figures 1 to 6.

It is of considerable interest that the stem of the stapedial artery forms an anastomosis, not with vascular remnants in the position of the dorsal part of the first aortic arch (as in the rat; Tandler, 1902), but with vessels which succeed the ventral part of that arch, namely, the ventral pharyngeal arteries (as in the rabbit; Fuchs, 1905). Unlike the corresponding vessel in the rat embryo, the dorsal or carotid end of the mandibular artery, which is the successor to the first aortic arch, gives rise to no appreciable branches and dwindles early. The major substance of the first or mandibular bar is supplied by the ventral pharyngeal artery (figs. 3, 4) throughout the branchial period, after which time the changed relations in the environment permit its distal end to be readily annexed by the stem of the stapedial artery (fig. 6; figs. 30–32, pl. 3).

At the height of its development (16 to 18 mm.) the stapedial artery has two primary divisions, the dorsal one passing far cranially to the supraorbital region in company with the ophthalmic branch of the fifth nerve, and the ventral one giving off two branches in association with the maxillary and mandibular roots of this nerve (fig. 7). During the 20-mm. stage the stem of the ophthalmic artery anastomoses with the supraorbital division of the stapedial (fig. 8) and eventually annexes the distal end of this artery to form its lacrimal branch (fig. 10; figs. 34–36, pl. 3). In like manner the external carotid artery, through its internal maxillary branch, annexes the maxillomandibular division of the stapedial artery at the mandibular nerve root to form the first part of the middle meningeal artery (figs. 8, 9). The second part of the middle meningeal represents the proximal part of the supraorbital division of the stapedial artery, which passes lateral to the Gasserian ganglion. Following these developments, the stem of the stapedial artery is interrupted at the stapes just distal to the original hyoid artery, which remains as the small caroticotympanic branch of the adult internal carotid; the remainder of the stapedial stem is the superior tympanic branch of the adult middle meningeal artery (fig. 8; fig. 35, pl. 3).

The formation of the definitive ophthalmic artery is both complicated and relatively late. The modifications of ophthalmic supply seen in the earlier stages of human embryos resemble those described in the rabbit by Fuchs (1905), but, in contrast, the late stages of the two species diverge markedly. The middle meningeal artery, representing the supraorbital

division of the stapedial artery, takes over most of the branches of the ophthalmic artery in the rabbit, whereas in man the ophthalmic artery annexes the distal end of the supraorbital division including all its orbital branches. Although not all complicated details in the formation of the ophthalmic artery were determined with certainty, essential features were revealed, and six stages are illustrated in figure 13.

For a relatively long period (4 to 18 mm.) the arteries to the human eye come from a part of the internal carotid which is considerably distal to the origin of the adult ophthalmic artery. In an early stage (3 to 4 mm.) the primitive internal carotid artery, near the point of its primary cranial and caudal divisions, gives off several short branches to the mesial face of the large optic vesicle (figs. 1, 2; figs. 38, 39, pl. 4), the base of which is supplied by a transitory (hypophthalmic) branch of the primitive maxillary artery (fig. 3*b;* fig. 44, pl. 5). Soon the primitive maxillary artery, confined medially along the neck of Rathke's pouch, loses all direct connection with the optic region (figs. 2, 13*a;* fig. 45, pl. 5). In embryos between 4 and 6 mm., most of the primitive eye, including the dorsal aspect of the optic vesicle, now undergoing differentiation, and the ectodermal lens pit, is supplied by a long carotid branch which arises opposite the posterior communicating artery and dips into the depression representing the fetal ocular cleft (figs. 3, 13*a*). In contrast with an opposing artery to be noted presently, the origin and destination of this artery, relative to the eye in all early stages, is primarily dorsal, although also caudal and lateral, and its main stem becomes the common temporal ciliary artery; it was named for this study the primitive dorsal ophthalmic artery. Its stem, which was represented in the preceding stage (figs. 1, 2), becomes even more prominent in the late branchial period (7 to 12 mm., fig. 4), at which time it takes a direct lateral course to the dorsal aspect of the optic cup and is lost in a manifold plexus now enveloping it (a primitive choriocapillaris, fig. 13*b*). Although this dorsal ophthalmic artery cannot, therefore, be traced into the outer end of the fetal ocular cleft (choroid fissure), as in the preceding stage, the plexus which it supplies, including an annular vessel around the periphery of the lens, is connected with a sinus-like vessel lying in the cleft mesial to the lens (primitive hyaloid vessel, fig. 13*b*)—a vessel which drains directly into the inferior maxillary veins. At this stage the more ventral, cranial, and mesial parts of the optic capillary mesh are supplied by another branch of the internal carotid which is here named the primitive ventral ophthalmic artery. It comes from the most distal and ventral segment of the internal carotid, opposite its anterior choroidal branch, which crosses the diencephalon (figs. 4, 13*b*). The definitive terms "ophthalmic" and "hyaloid" artery used by earlier writers in reference to vessels of this stage of the branchial period are therefore not justified. In the early postbranchial phase (between 12 and 14 mm.) the optic stalk further elongates, shifting the eye away from the brain wall and the internal carotid artery. As a result, both primitive ophthalmic arteries, dorsal and ventral, are drawn out in length; although their point of origin relative to the eye and the direction of their course is changed, the terminal area of each remains the same (compare figs. 4 and 5). Meanwhile the primitive dorsal ophthalmic artery has developed a direct branch to the lens through the mesial end of the fetal ocular cleft; this is the hyaloid artery, and the distal end of its parent artery is recognizable as the common temporal ciliary artery (figs. 5, 13*c*). Between 16 and 18 mm. these ocular branches are transferred to the permanent stem of the ophthalmic artery, which was first identified late in this stage (figs. 7, 13*d*). It is apparent that the ophthalmic artery, with its internal carotid origin caudal to the optic stalk and opposite Rathke's pouch, has finally reached this adult position by a considerable caudal (or proximal) migration along the carotid of the primitive dorsal ophthalmic artery which it represents; as noted above, this primitive artery arises opposite the posterior communicating artery, well cranial to the optic stalk. There is evidence that this movement is permitted by a succession of anastomotic loops (Streeter's "anastomotic progression") from the carotid during the postbranchial stage, when the expanding brain and cerebral arteries become more and more withdrawn from the developing optic nerve and eye. The condensation of mesenchyme in the position of the orbital roof, at the future optic foramen, completes the process of relegating the ophthalmic artery to its permanent carotid origin below the level of this structure. At this time the stem of the ophthalmic artery annexes the distal end of the attenuated primitive ventral ophthalmic artery, coming from the most dis-

tal segment of the internal carotid (opposite the anterior choroidal artery); in this manner is formed the common nasal ciliary artery (fig. 33, pl. 3). By the 20-mm. stage, therefore, all the ocular branches of the ophthalmic artery have been established.

The orbital branches which form the most conspicuous part of the adult ophthalmic artery are derivatives of the supraorbital division of the stapedial artery (fig. 13*d*). Through the formation of an arterial ring around the optic nerve during the 20- to 24-mm. stage (fig. 13*e*) and the subsequent interruption of a ventral segment of this ring (fig. 13*f*), representing usually a part of the secondarily formed common nasal ciliary branch of the ophthalmic stem, the ophthalmic artery is allowed to pass from the ventral to the dorsal side of the nerve, as in the adult, and becomes continuous with the supraorbital division of the stapedial artery. Sometime between 24 and 40 mm., the first, strictly orbital segment of the original supraorbital division, now part of the middle meningeal artery, becomes the stem of the lacrimal branch of the ophthalmic artery. Toward the end of this period, an interruption or dwindling of the original supraorbital division at the orbital margin, that is, between its proximal and distal parts, which now belong to the middle meningeal and lacrimal arteries respectively, leaves the ophthalmic artery recognizably identical with the adult configuration (fig. 13*e, f;* figs. 35, 36, pl. 3). The embryonic connection between the ophthalmic and stapedial arteries is frequently represented in the adult by a branch of variable size connecting the ophthalmic (commonly its lacrimal branch) and middle meningeal arteries at the lateral end of the sphenoid fissure.

Finally there is described the development of a conspicuous temporary artery which, in reference to the human embryo at least, has heretofore received no attention in the literature. This artery contributes extensively toward the formation of the bilateral longitudinal neural arteries which join to form the basilar artery, and because of its position has here been named the primitive trigeminal artery. The first representation of this trigeminal vessel was found in a 20-somite embryo of 3 mm., in which there are two definite branches from the paired (dorsal) aorta at its point of junction with the first aortic arch (fig. 37, pl. 4). One of these is the primitive internal carotid artery, which extends to the optic vesicle at the forebrain. The other is the primitive trigeminal artery, which leaves the aorta just caudal to the carotid branch, and arches dorsally toward the fifth nerve ganglion (fig. 43, pl. 5). The trigeminal artery at this stage supplies the cranial end of the primordial hindbrain channel, from whose proliferating endothelium the arteries (for instance, those which precede the basilar) and veins of the hindbrain wall are subsequently derived.

In the next stage (4 to 5 mm.) the bilateral longitudinal neural arteries, which are destined to form the basilar artery, emerge as derivatives of a plexus which earlier resulted from differentiation of the primordial hindbrain channel (figs. 38, 39, pl. 4; fig. 1). The cranial end of each neural artery is supplied by the trigeminal artery, which, following the involution of the first two aortic arches, is now considered a branch of the primitive internal carotid artery. Development of the diencephalic and mesencephalic regions at this time is accompanied by an extension of the primary caudal division of the internal carotid artery (fig. 1). Not until it has developed a secondary anastomosis with the cranial end of the neural artery, however, does the caudal division of the carotid become the definitive posterior communicating artery (fig. 2). Until this time the trigeminal branch of the internal carotid is the major source of blood to the longitudinal neural artery of the hindbrain, the caudal connections of which with the transitory hypoglossal artery and the first cervical segmental artery are typically small. Subsequently, the bilateral neural arteries consolidate to form the basilar artery. Concomitantly or soon thereafter, the posterior communicating artery takes over from the trigeminal artery the role of supply to the arteries of the hindbrain during the remainder of the branchial period.

Following the establishment of the posterior communicating artery (an important channel in the embryo in contrast with its subsidiary role in the adult circle of Willis), the trigeminal artery usually dwindles. In certain embryos, however, the primitive trigeminal branch persists for varying periods after the formation of the posterior communicating and basilar arteries, producing a prominent caroticobasilar anastomosis (figs. 40–42, pl. 4). In such cases the posterior communicating artery, in the terminal part connecting it with the basilar artery, was found to be relatively small. No large trigeminal or caroticobasilar anastomosis was identified beyond the early post-

branchial period, when the formation of the vertebral artery relieves the internal carotid of its earlier role of supply to all the arteries of the hindbrain. Other factors contribute to the elimination of the trigeminal anastomosis; namely, unequal environmental growth with consequent shifting in the relative position of the carotid and basilar ends of the anastomosis, which opposes its course to that of the current in the internal carotid; and the interposition of the basal sphenoid cartilage between the carotid and basilar ends during the 12- to 20-mm. stages. Interrupted remnants, particularly of the basilar end of the anastomosis, were identified in several embryos up to 40 mm. (figs. 47, 48, pl. 5). The occasional persistence of the primitive trigeminal artery in the adult, like that of several other arteries which serve temporary embryonic needs, may result in a significant adult anomaly not infrequently reported in the literature—an anastomotic branch of considerable size between the basilar artery and the intracavernous part of the internal carotid.

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## LIST OF ABBREVIATIONS IN FIGURES

ant. card. v., anterior cardinal vein
ant. cerebr. a., anterior cerebral artery
ant. choroid. a., anterior choroidal artery
ant. commun. a., anterior communicating artery
ant. ethm. a., anterior ethmoid artery
ant. inf. cerebel. a., anterior inferior cerebellar artery
ant. perfor. subst., anterior perforated substance
ant. spin. a., anterior spinal artery
aort. arch, aortic arch
arter. tr., arterial trunk
axil. a., axillary artery
bas. a., basilar artery
bilat. long. neur. a., bilateral longitudinal neural artery
brach. a., brachial artery
carot.-bas. anast., caroticobasilar anastomosis
carot.-tymp. a., caroticotympanic artery
caud. divis., caudal division
cent. a. of ret., central artery of retina
choroid. plex., choroidal plexus
chord. tymp. n., chorda tympani nerve
com. nas. cil. a., common nasal ciliary artery
com. temp. cil. a., common temporal ciliary artery
cran. divis., cranial division
dienceph. a., diencephalic artery
dist., distal
duct. art., ductus arteriosus
ext. carot. a., external carotid artery
ext. max. a., external maxillary artery
fetal ocul. cleft, fetal ocular cleft
first cerv. segm. a., first cervical segmental artery
first cerv. mesoblast. segm., first cervical mesoblastic segment
front. a. (n.), frontal artery (nerve)
hyal. a., hyaloid artery
inf. alveol. a., inferior alveolar artery
inf. hypoph. a., inferior hypophyseal artery
infraorb. a. (n.), infraorbital artery (nerve)
innom. a., innominate artery
int. aud. a., internal auditory artery
int. carot. a., internal carotid artery
int. mam. a., internal mammillary artery
int. max. a., internal maxillary artery
lacrim. a., lacrimal artery
ling. a., lingual artery
mand. a., mandibular artery
max.-mand. div., maxillomandibular division
med. a. of corp. cal., median artery of corpus callosum
med. str. a. (recur. a.), medial striate artery (recurrent artery of Heubner)
mesenceph. a., mesencephalic artery
metenceph. a., metencephalic artery
mid. cerebr. a., middle cerebral artery
mid. mening. a., middle meningeal artery
nas. fossa, nasal fossa
nasocil. n., nasociliary nerve
occip. a., occipital artery
olf. area, olfactory area
ophth. a., ophthalmic artery
opt. ves., optic vesicle
ot. ves., otic vesicle
paired aort., paired aorta
pharyng. bar, pharyngeal bar
post. auric. a., posterior auricular artery
post. cerebr. a., posterior cerebral artery
post. choroid. a., posterior choroidal artery
post. commun. a., posterior communicating artery
post. inf. cerebel. a., posterior inferior cerebellar artery
post. spin. a., posterior spinal artery
prim. head v., primary head vein
primit. otic a., primitive otic artery
primit. dors. ophth. a., primitive dorsal ophthalmic artery
primit. hypoglos. a., primitive hypoglossal artery
primit. lat. bas.-vert. anast., primitive lateral basilovertebral anastomosis
primit. max. a., primitive maxillary artery
primit. olf. a., primitive olfactory artery
primit. trigem. a., primitive trigeminal artery
primit. vent. ophth. a., primitive ventral ophthalmic artery
primord. hindbr. channel, primordial hindbrain channel

primord., primordium
pulm. a., pulmonary artery
remn., remnant
second. anast., secondary anastomosis
som., somite
staped. a., stapedial artery
subcl. a., subclavian artery
supfic. petros. n., superficial petrosal nerve
supfic. temp. a., superficial temporal artery
sup. cerebel. a., superior cerebellar artery
sup. hypoph. a., superior hypophyseal artery
sup. thyr. a., superior thyroid artery
sup. tymp. a., superior tympanic artery
supraorb. a. (n.), supraorbital artery (nerve)
supraorb. div., supraorbital division
thyr.-cerv. tr., thyrocervical trunk
tymp. n., tympanic nerve
ventr., ventricle
vent. pharyng. a., ventral pharyngeal artery
vert. a., vertebral artery

Adaptation of the cranial arteries to growth of the brain and head from 4 mm. to birth. (Modified from text figs. 1–11.)

FIG. 14. (Embryo no. 588, 4 mm.) The primitive internal carotid supplies the forebrain through its cranial and caudal divisions and its primitive maxillary branch, and supplies the hindbrain through the primitive trigeminal branch which joins the cranial end of the bilateral neural artery. The caudal end of the neural artery is fed by the primitive hypoglossal and first cervical segmental arteries. Hyoid and mandibular arteries have succeeded the first two aortic arches.

FIG. 15. (Embryo no. 2841, 5.3 mm., supplemented by no. 3960, 5.5 mm.) The caudal division of the internal carotid has become the definitive posterior communicating artery following anastomosis with the bilateral neural artery, which is consolidating with its mate of the opposite side to form the basilar artery. The primitive trigeminal and hypoglossal arteries are dwindling. The substance of the first two pharyngeal bars is supplied by the ventral pharyngeal artery reinforced by the hyoid artery.

FIG. 16. (Embryo no. 163, 9 mm.) The vertebral artery is being formed by transverse anastomoses between each of the upper seven cervical segmental arteries, but all cerebral arteries are still supplied primarily by the internal carotid. The stem of the hyoid artery lies at the cranial level of the hyoid bar, and its course is more caudal, corresponding to the caudal swing of the seventh nerve. The third pharyngeal bar with its nervous and arterial components is no longer a separate entity. The ventral pharyngeal artery, skirting the mandibular end of the chorda tympani nerve, terminates at the mandibular nerve root. In addition to the primitive dorsal ophthalmic artery of preceding stages, a primitive ventral artery supplies the plexus investing the optic cup and lens.

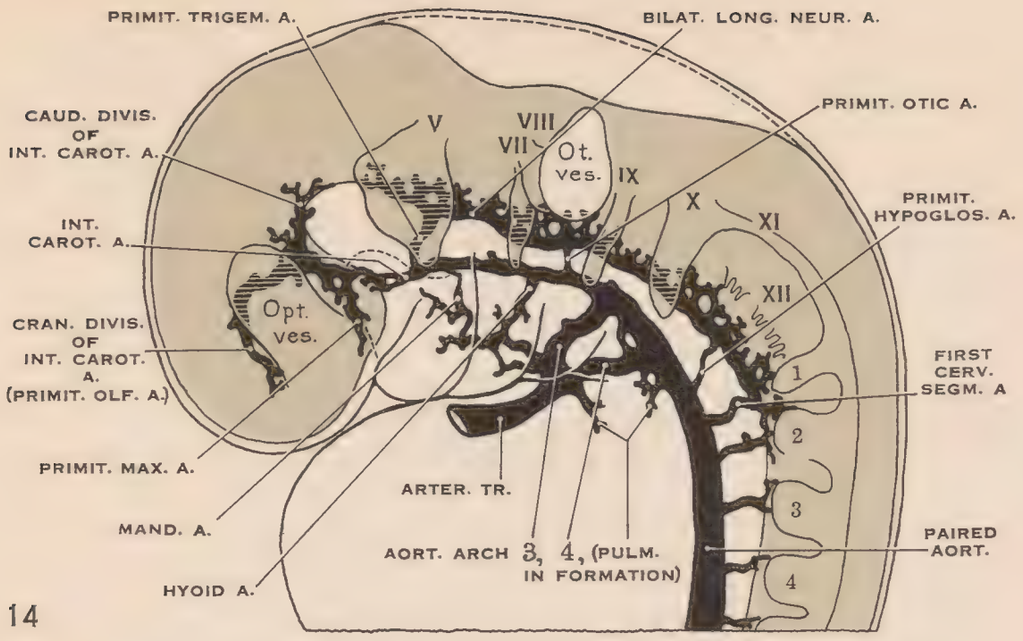
FIG. 17. (Embryo no. 1771, 12.5 mm.; details of stapedial artery from nos. 940 and 6258, each 14 mm.) In this early postbranchial phase, the hyoid artery, now no longer readily identified with its corresponding pharyngeal bar, gives off the stapedial branch, which descends into the mandibular substance and joins the distal remnant of the ventral pharyngeal artery, whose proximal end represents the stem of the external carotid. Dwindling of the paired aorta between the third and fourth aortic arches permits recognition of the common carotid, and the subclavian origin of the vertebral artery is being shifted cranially. The primitive dorsal ophthalmic artery has definitive hyaloid and temporal ciliary branches.

FIG. 18. (Embryo no. 1390, 18 mm.) The stem of origin of most cerebral arteries and external carotid branches is typically adult. The primitive olfactory artery, which constituted the terminal end of the cranial carotid division in all earlier stages, is dwindling in favor of its anterior cerebral collateral. The stapedial artery has two divisions, comprising three branches, which supply the orbital, maxillary, and mandibular regions. The adult stem of the ophthalmic artery is present for the first time, having annexed the ocular branches of the dwindling primitive dorsal and ventral ophthalmic arteries. The vertebral artery is fully formed, and its parent subclavian artery now arises opposite the pulmonary arch.

FIG. 19. (Embryo no. 632, 24 mm.) The expanding cerebrum is responsible for the medial prolongation of the anterior cerebral arteries, which are now joined by the anterior communicating artery. The stem of the stapedial artery has dwindled following two secondary anastomoses: the ophthalmic artery has annexed its supraorbital division and the internal maxillary its maxillomandibular division, permitting the last named to become recognizable as the stem of the middle meningeal artery.

FIG. 20. (Embryo no. 886, 43 mm.; details of certain arteries from no. 6203, 39 mm.) With further development of the cerebrum, the posterior communicating branch of the carotid has nearly completed its swing from the caudocranial direction to the reverse. The stems of the anterior and posterior inferior cerebellar arteries, long concealed in remnants of the primitive basilovertebral anastomosis (figs. 16, 17), are recognizable and terminate in the choroid plexus of the fourth ventricle. The large choroid plexus of the lateral ventricle is supplied not only by the anterior and posterior choroidal arteries, but also by a temporary primitive branch of the anterior cerebral artery at the foramen of Monro. The ophthalmic artery has acquired its lacrimal branch, which represents part of the supraorbital division of the stapedial artery; the remainder of this division is incorporated in the middle meningeal artery.

FIG. 21. The newborn (or adult) configuration. Note that the only essential difference between the adult cerebral arteries and those of the preceding figure is dependent upon the ultimate predominance of the cerebral and cerebellar hemispheres. The formation of the corpus callosum and fornix has intervened between the anterior cerebral artery and the diencephalon so that that artery no longer supplies the choroid plexus. The posterior cerebral artery has emerged as a development of one of the diencephalic or mesencephalic branches, which are relatively reduced in the adult. In like manner, the anterior and posterior inferior cerebellar arteries become definitive and supply the cerebellar choroid plexus merely en route to their adult destination. The three branches of the temporary stapedial artery are recognizable as the supraorbital, infraorbital, and inferior alveolar arteries.

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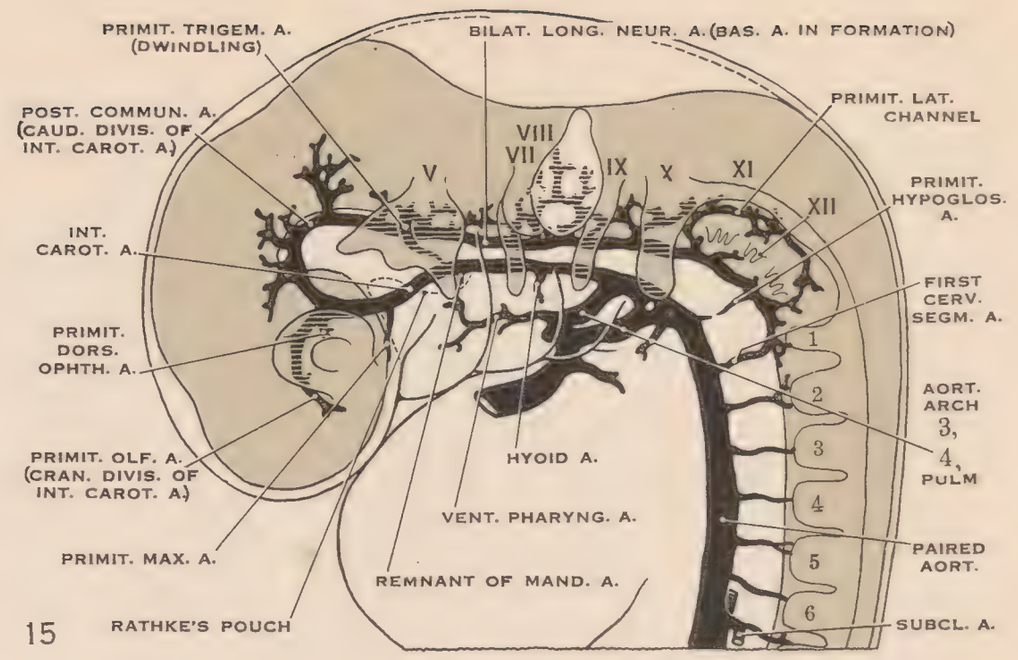


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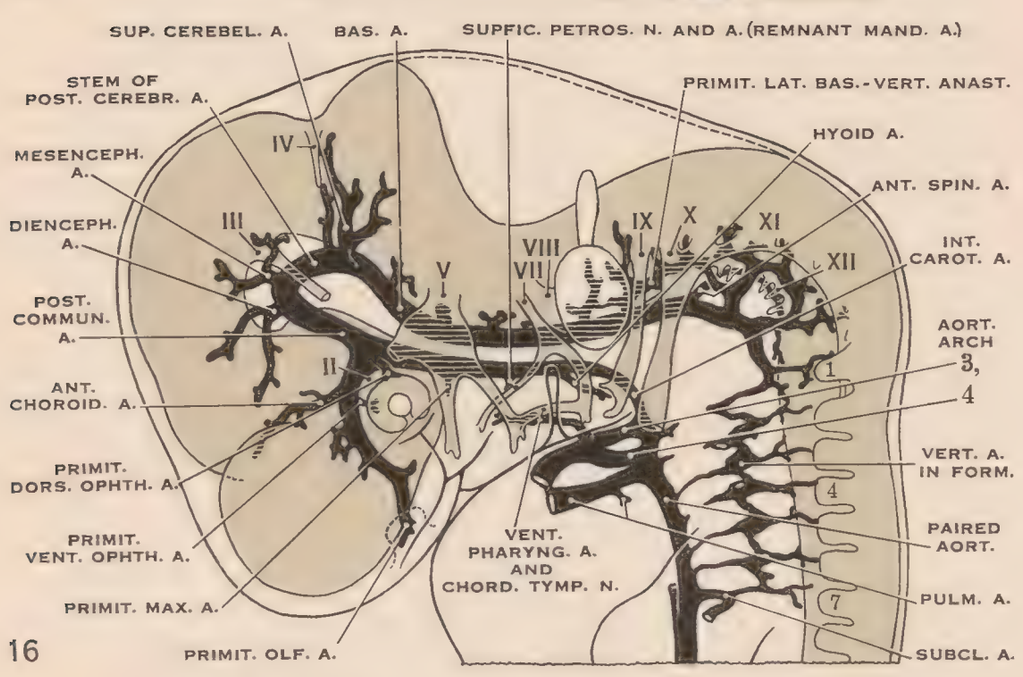


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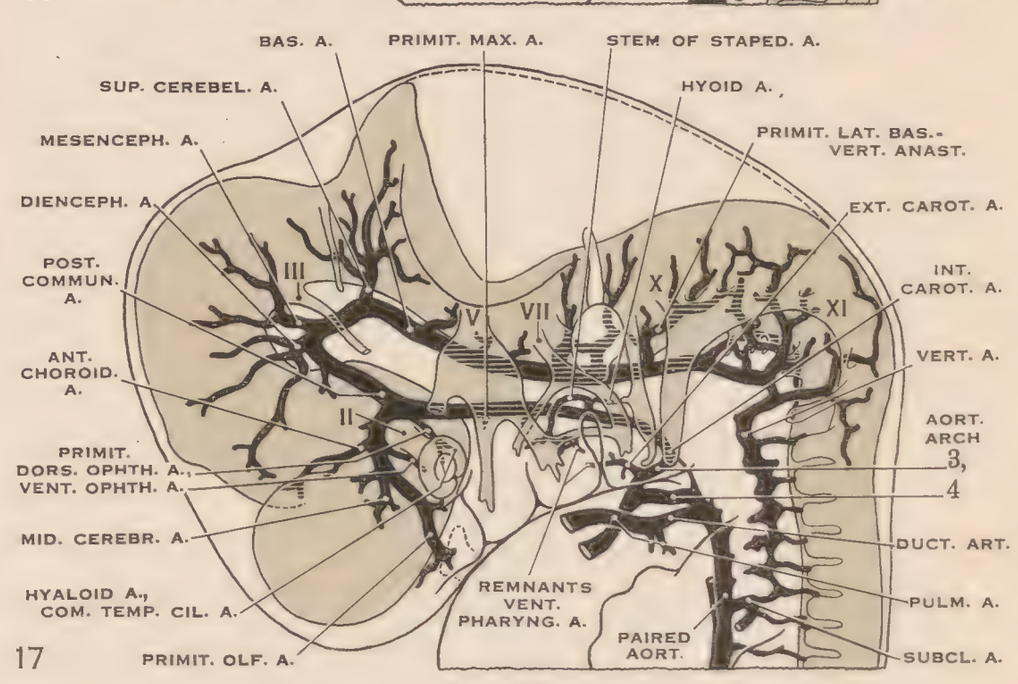


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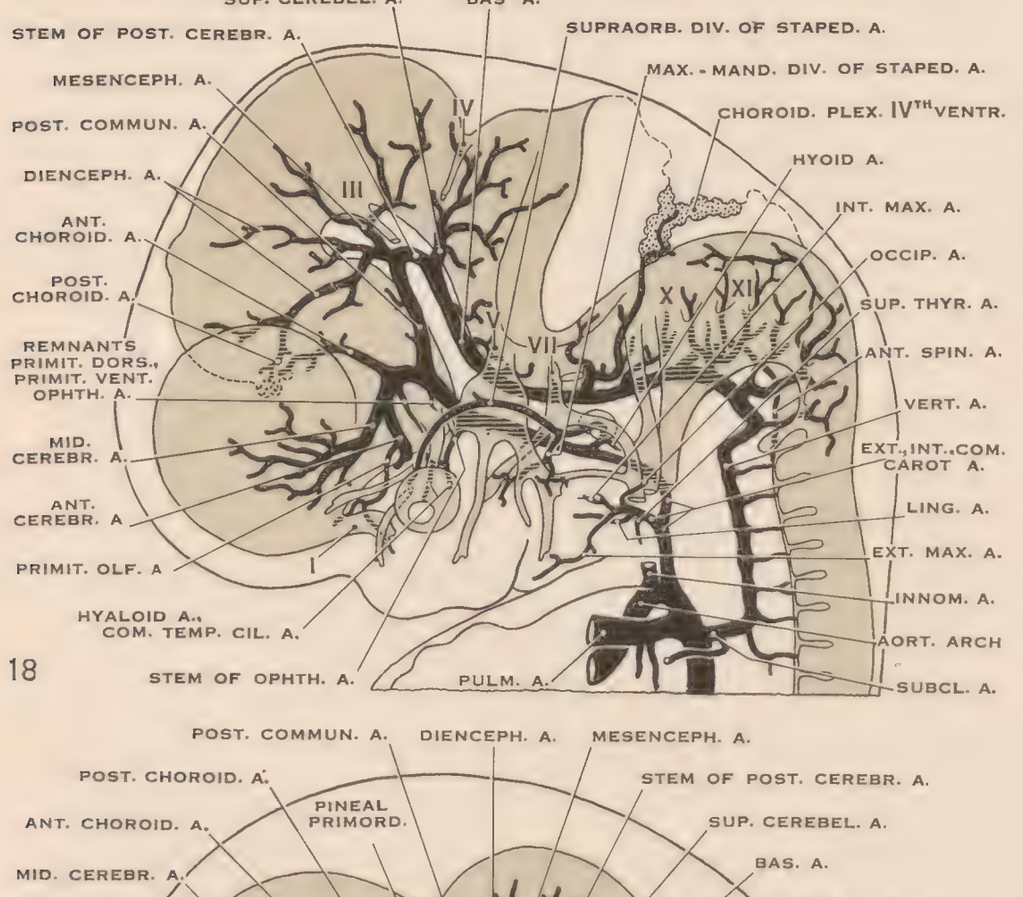


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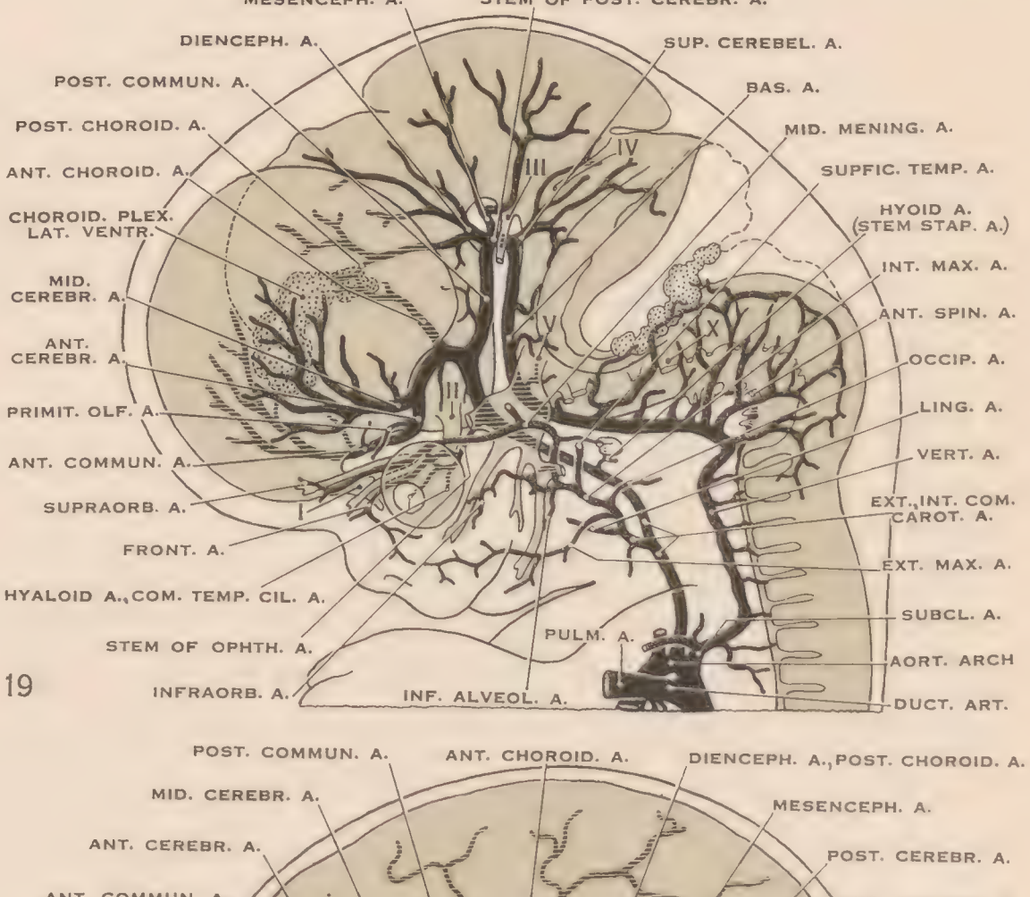


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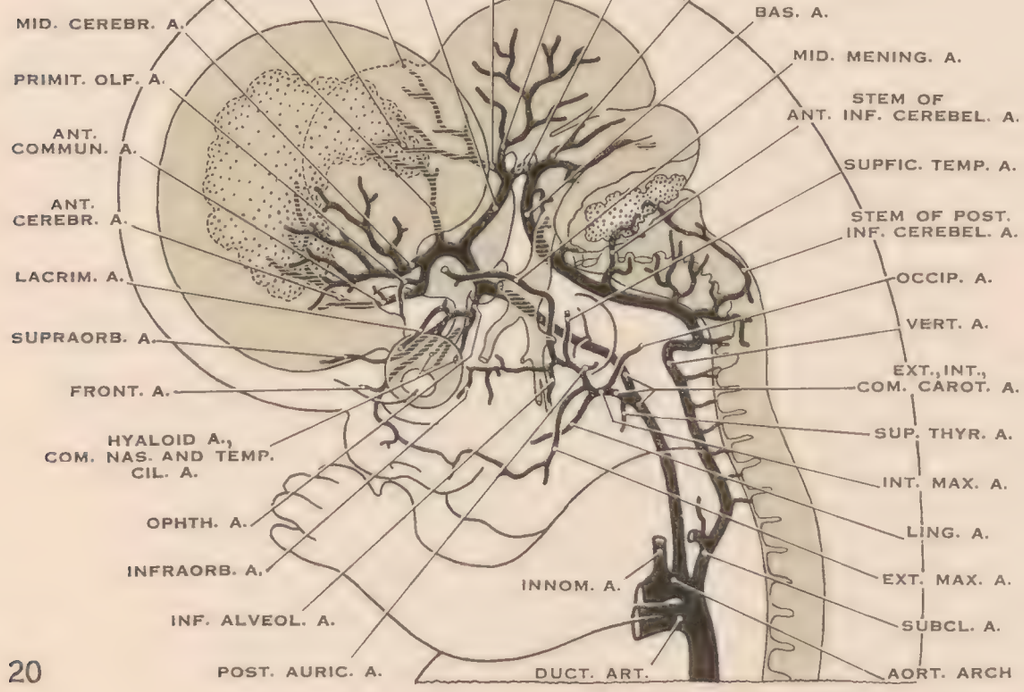


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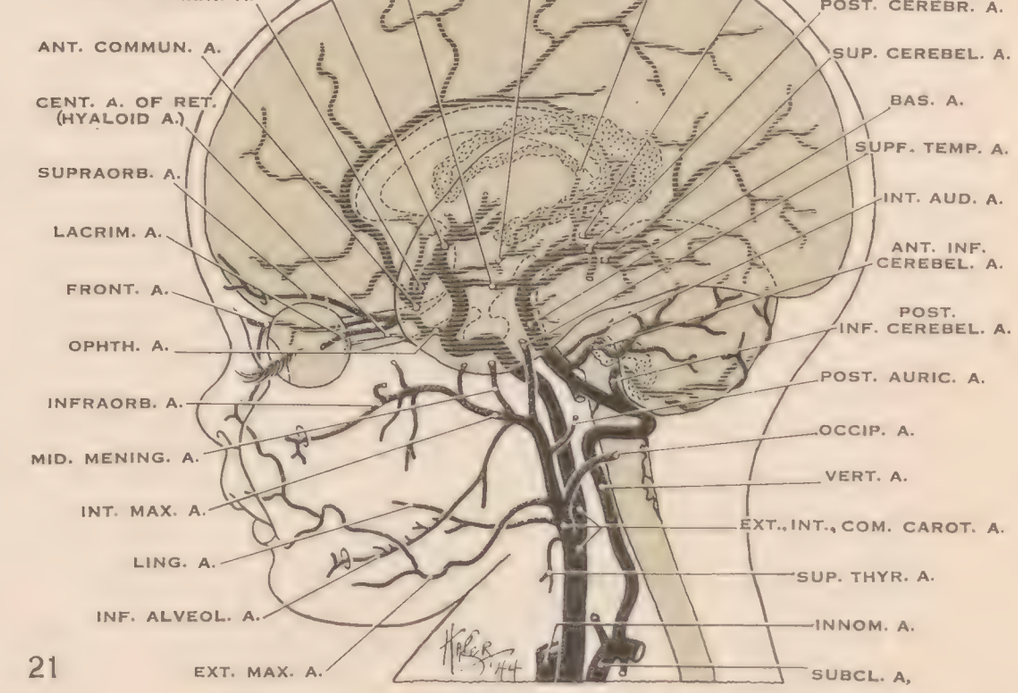


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Adaptation of the cerebral arteries to growth of the brain from 4 mm. to birth, and the formation of the circle of Willis. With one exception (fig. 24), these are the same embryos shown in plate 1 and are viewed from below so that the right side of the embryo is at the observer's left. (Modified from text figs. 1–11.)

FIG. 22. (Embryo no. 588, 4 mm.) Note that the internal carotid divides before reaching the forebrain; the dorsolateral branch is the temporary trigeminal artery, and the medioventral division is the permanent prolongation of the carotid skirting the margins of Rathke's pouch. The trigeminal artery supplies the bilateral longitudinal neural artery, and is reinforced by smaller presegmental branches of the carotid and paired aorta in the otic and hypoglossal regions.

FIG. 23. (Embryo no. 2841, 5.3 mm., supplemented by no. 3960, 5.5 mm.) The bilateral longitudinal neural arteries are consolidating to form the basilar artery and each is supplied by the new posterior communicating artery, which represents the caudal division of the carotid; the primitive trigeminal artery is dwindling. The cranial division of the carotid constitutes a primitive olfactory artery terminating at the olfactory plate.

FIG. 24. (Embryo no. 617, 7 mm., of the same stage as that shown in fig. 16, pl. 1.) The basilar artery is complete but irregular, with island formations. It is paralleled on either side by more or less continuous remnants of a channel (the basilovertebral anastomosis) which traverses the area between the mesial and lateral components of the sixth to twelfth nerve roots. The caudal ends of the posterior communicating arteries, terminating in the basilar artery, constitute the first parts of the posterior cerebrals.

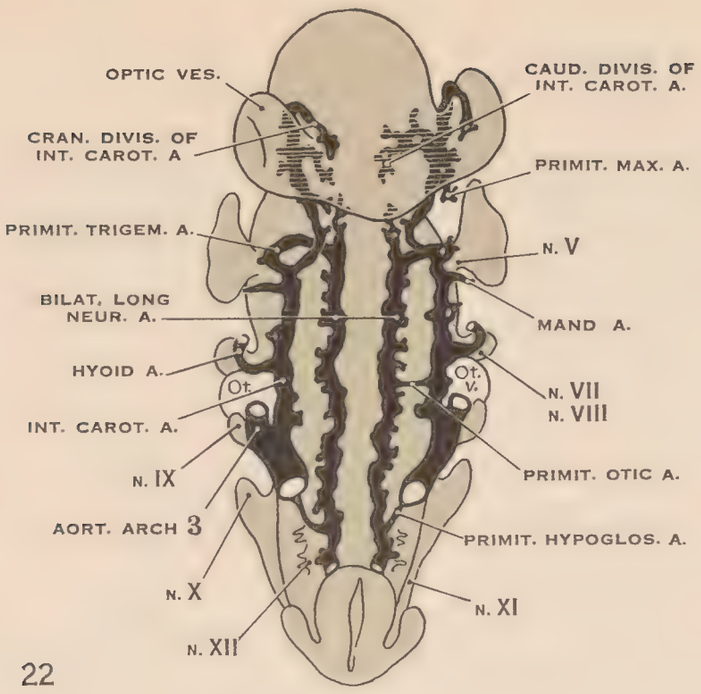
FIG. 25. (Embryo no. 1771, 12.5 mm.) The primitive olfactory artery has two branches, the original vessel terminating at the olfactory pit and a mesial branch going to the region of the primordial olfactory nerve root. Certain branches of the basilar and vertebral arteries are emerging from components of the basilovertebral anastomosis. The arterial ring around the sixth nerve, for instance, explains the variable origin of the basilar branches in this region during later stages and in the adult.

FIG. 26. (Embryo no. 1390, 18 mm.) The primitive olfactory artery supplies the nasal fossa through branches accompanying the olfactory root; but the artery is already overshadowed by its anterior cerebral collateral, which extends to a plexus at the mid-line. Note the different origins of the anterior inferior cerebellar and internal auditory arteries on the two sides in relation to the sixth nerve, and compare with figures 25 and 28. The new adult stem of the ophthalmic artery has annexed the primitive dorsal and ventral ophthalmic arteries which supplied the optic cup and lens in earlier stages.

FIG. 27. (Embryo no. 632, 24 mm.) The circle of Willis is complete with the formation of the anterior communicating artery, which gives off a median branch supplying the commissural plate. Remnants of the primitive olfactory artery are incorporated in striate branches of the anterior cerebral artery which penetrate the anterior perforated substance.

FIG. 28. (Embryo no. 886, 43 mm.) In embryos less than two months old, the adult configuration of the circle of Willis and its radiating branches has become readily recognizable in the ventral view of the brain, as a result of the expansion of the cerebral hemispheres and the accompanying caudal swing of the posterior communicating artery. This artery remains relatively large during fetal life, preceding the late completion of its collateral posterior cerebral branch, which in most adult cases is supplied more by the basilar than by the internal carotid artery.

FIG. 29. Newborn configuration. Note that the origin of the anterior cerebral artery, which, in the adult, is commonly hidden by the parent carotid and its middle cerebral branch when the circle of Willis is viewed *in situ,* is distal to that of the anterior choroidal and posterior communicating arteries. The relation of these arteries to each other is clear when this figure is compared with the embryo. Frequent variation and asymmetry, in the arteries forming the posterior half of the circle of Willis, is the result of an incomplete transition to the adult predominance of the basilar over the internal carotid in supply to the posterior cerebral artery. The medial striate branch of the anterior cerebral, which varies considerably in size, is potentially significant.

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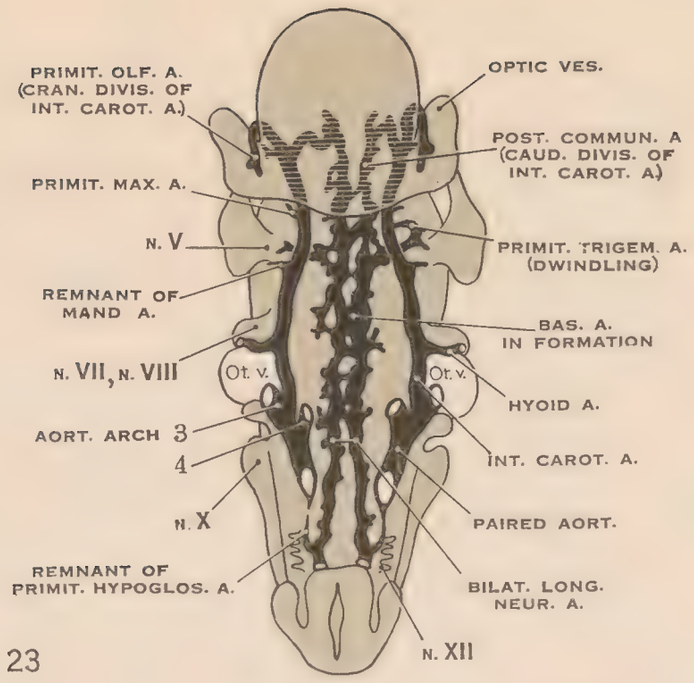


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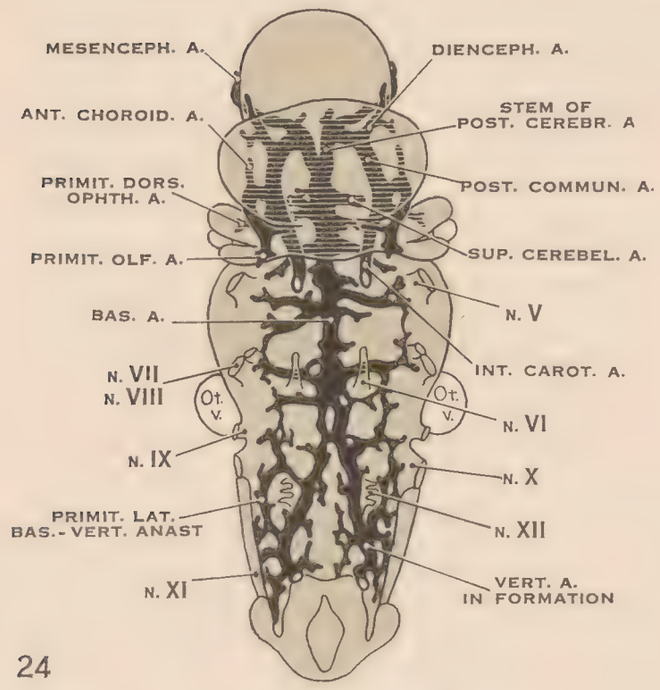


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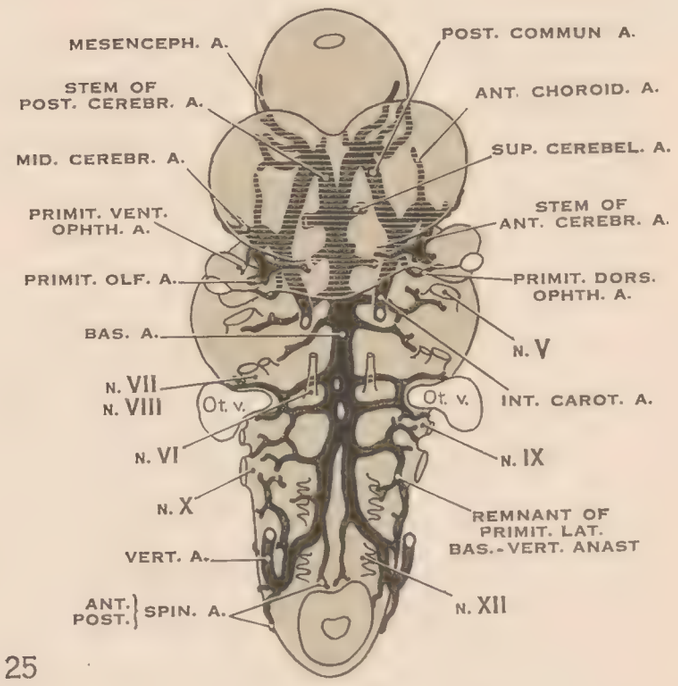


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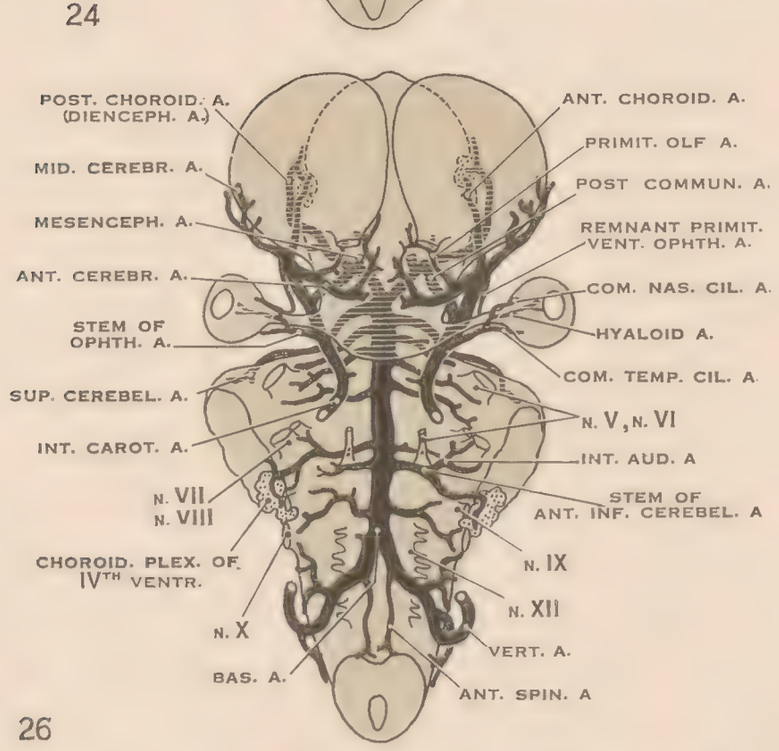


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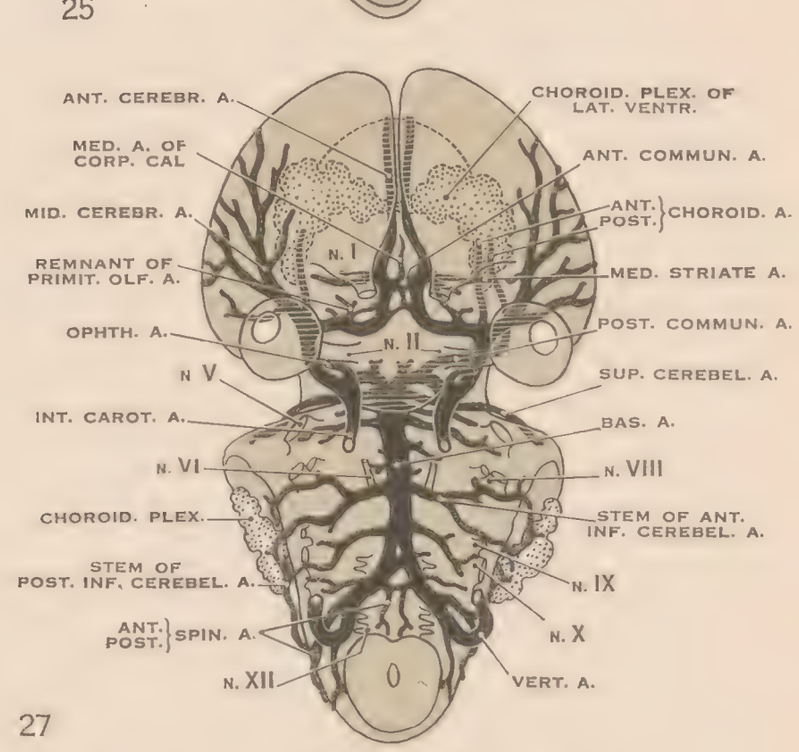


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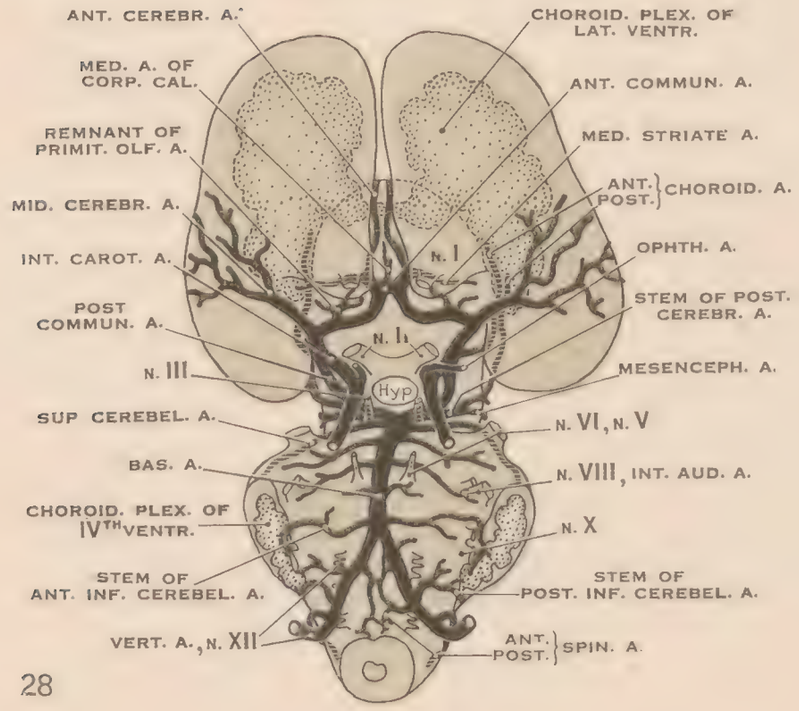


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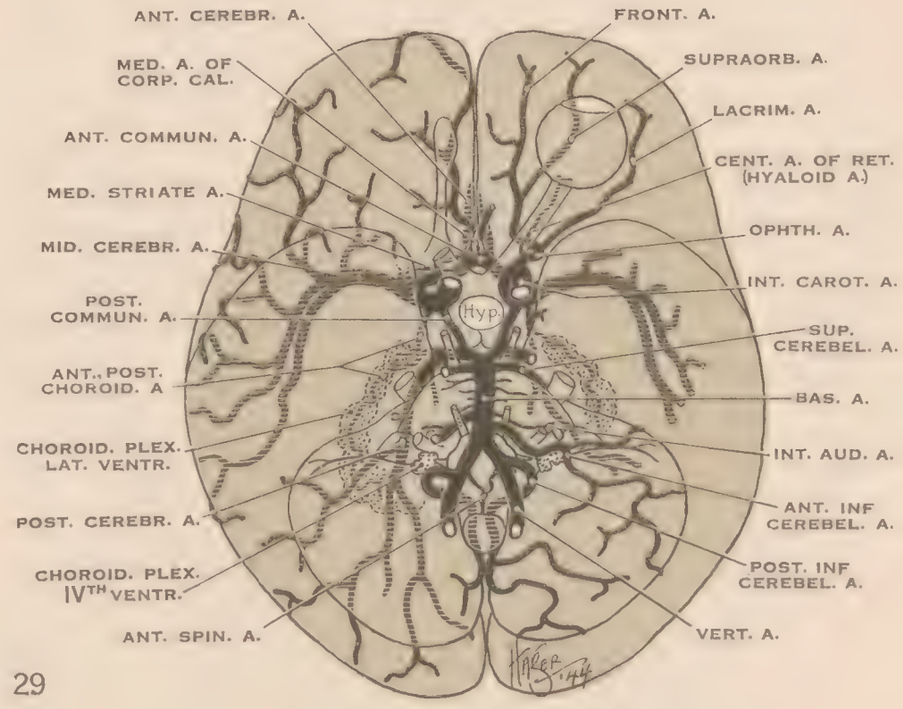


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Partial horizontal reconstructions of 7 embryos from 4 to 40 mm. (ventral view), to illustrate the environmental changes leading to the formation of the stapedial artery and the incorporation of its supraorbital division in the ophthalmic artery.

FIG. 30. (No. 2841, 5.3 mm. See lateral view in text fig. 2*a*.) In this embryo of the early "branchial" phase, there is a regular conformation of the cranial nerve roots and aortic arches to each pharyngeal bar and corresponding pouch. The area formerly occupied by the first and second aortic arches is now traversed by two vessels: the hyoid artery, representing the dorsal end of the second arch, and the ventral pharyngeal artery (terminating at the mandibular nerve root), in the general position of the ventral ends of both arches. Note that the dorsal end of the first aortic arch is represented by only a small remnant of the mandibular artery. ×30.

FIG. 31. (No. 617, 7 mm. See lateral view of same stage in text fig. 4*a*.) The third pharyngeal bar has lost definition and, coincidently with the development of the maxillary process and several collateral nerve roots, the fifth to the tenth cranial nerves appear to be bunched together, and the seventh nerve has begun to swing caudally. Note that the carotid origin of the hyoid artery, near the caudal border of the corresponding pharyngeal bar in the previous stage (fig. 30), is now at the level of the cranial border. ×21.6.

FIG. 32. (No. 940, 14 mm.; distal connections of stapedial artery superimposed from another 14-mm. embryo, no. 6258. See lateral view of this reconstruction in text fig. 6.) This embryo is emerging from the "branchial" phase, and the carotid stem of the hyoid artery no longer appears to be within the hyoid bar, but is cranial to the first pharyngeal cleft. These altered relations readily permit the stapedial collateral of the hyoid artery to annex the distal remnant of the ventral pharyngeal artery at the mandibular nerve root, thereby forming the primary maxillomandibular division of the stapedial artery. ×15.

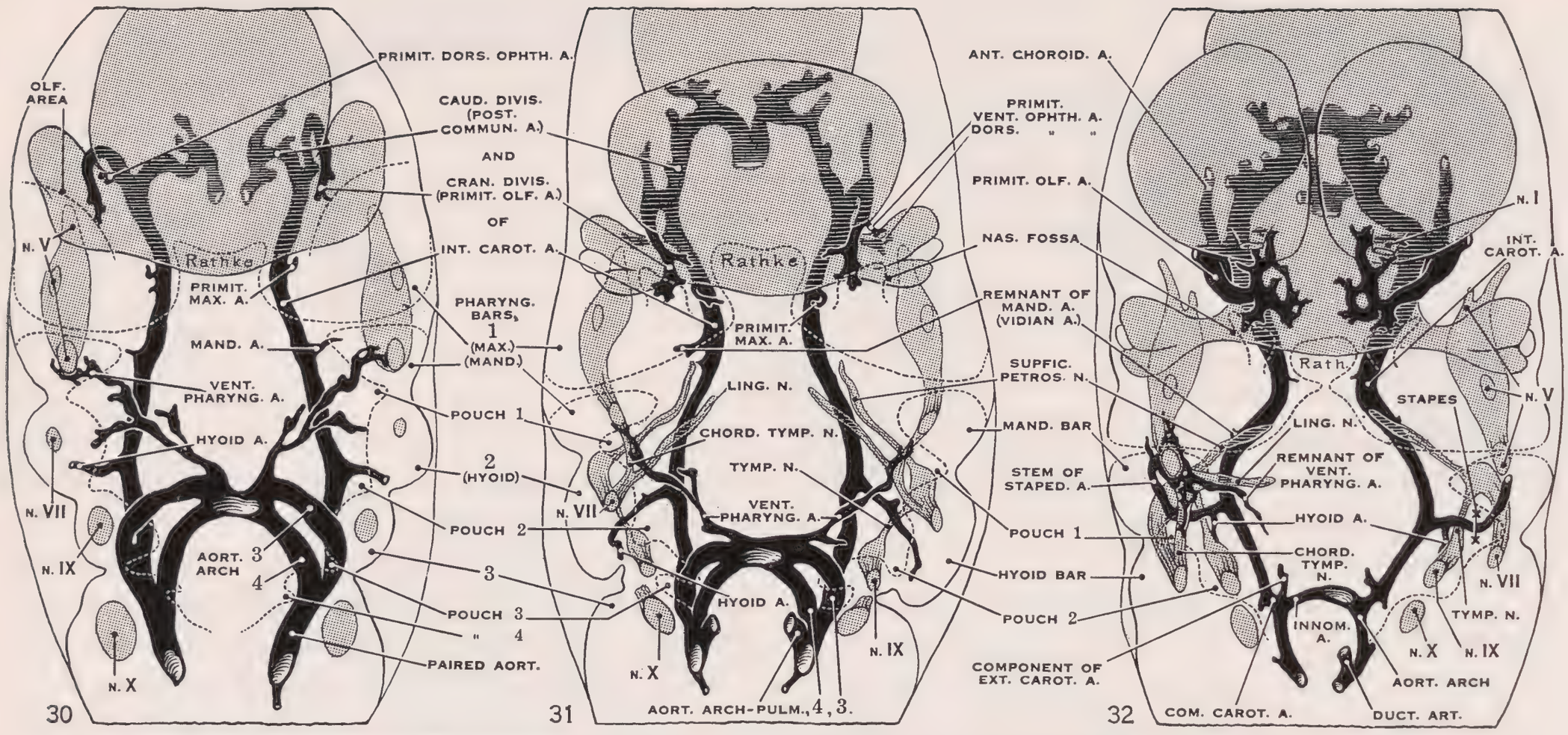
FIG. 33. (No. 1390, 18 mm. Other illustrations in text fig. 7.) The upper supraorbital division of the stapedial artery, whose stem was formed in the preceding stage (fig. 32), passes into the primitive orbit as far as the division of the ophthalmic nerve root. The stem of the ophthalmic artery (earlier stages shown in text fig. 13) has formed two of its primary ocular branches from the primitive ophthalmic arteries, and is about to annex the third, as shown in the diagrammatic insert. ×15.

FIG. 34. (No. 6202, 21 mm.) The supraorbital division of the stapedial artery has formed two orbital branches accompanying the frontal and nasociliary branches of the ophthalmic nerve. The artery could not be traced into the orbit on the left side of the embryo; on the right, however, it has joined the stem of the opththalmic artery dorsal to the optic nerve and has, therefore, completed a developmental phase shown in the generally more advanced embryo of the succeeding figure 35. ×15.

FIG. 35. (No. 632, 24 mm. See lateral view in text fig. 8*a*.) This embryo shows how the ophthalmic artery gains the dorsal side of the optic nerve and acquires its orbital branches. The arterial ring surrounding the optic nerve permits the supraorbital division of the stapedial artery, which now has three branches coinciding with the subdivision of the frontal nerve, to be annexed by the ophthalmic artery lying below the optic nerve. Another arterial ring, which transfers the internal maxillary artery to the outer side of the mandibular nerve root, is seen on the left side of the embryo. The stem of the stapedial artery has been interrupted just distal to its parent hyoid artery, and the maxillomandibular division of the stapedial has become the stem of the definitive middle meningeal artery. ×12.

FIG. 36. (No. 6203, 39 mm.) The adult configuration of the ophthalmic artery is recognizable on the left side of this embryo, and the stapedial artery has been completely taken over by the internal maxillary and ophthalmic arteries. On the right side of the embryo, the arterial ring around the optic nerve persists; on the left, interruption of the ring allows the ophthalmic artery to pass dorsal to the nerve. On both sides the ophthalmic artery has retained and augmented the orbital end of the supraorbital division of the stapedial to form its lacrimal branch. The remainder of the supraorbital division, lateral to the Gasserian ganglion, is incorporated in the middle meningeal artery. ×10.8.

These seven embryos also illustrate the development of the anterior cerebral artery. This forms as a collateral of the primitive olfactory artery (figs. 30–33), components of which are ultimately incorporated in the adult striate branches of the anterior cerebral (figs. 33–35). The medial striate artery (figs. 35, 36) is relatively constant in the adult; the variable striate branch found on the first part of the adult anterior cerebral is a remnant of the original stem of the primitive olfactory artery (right side of embryo, fig. 36).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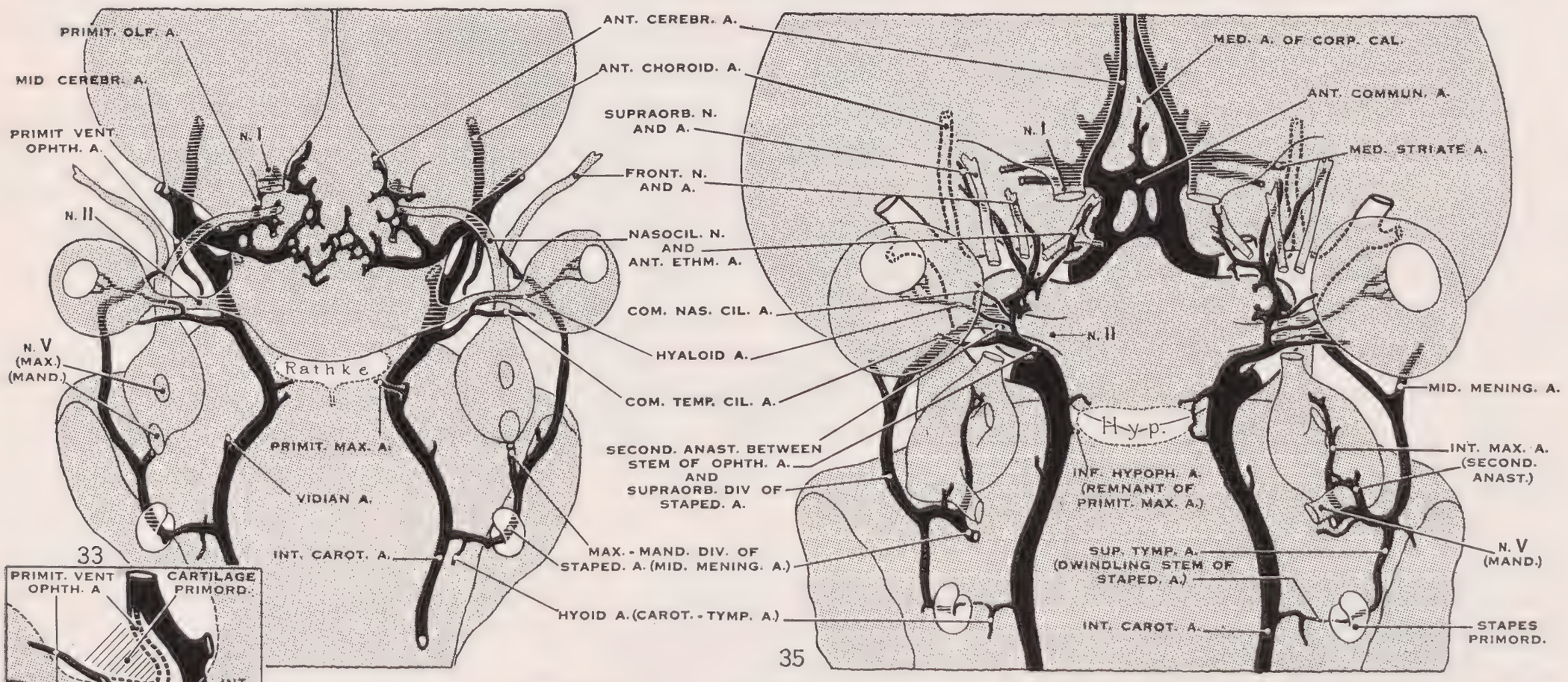
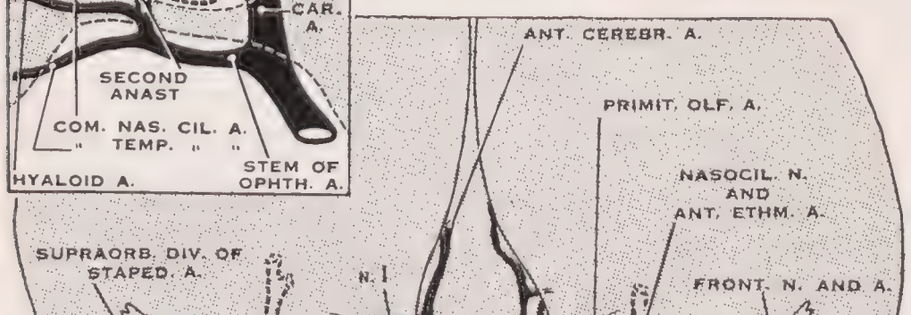
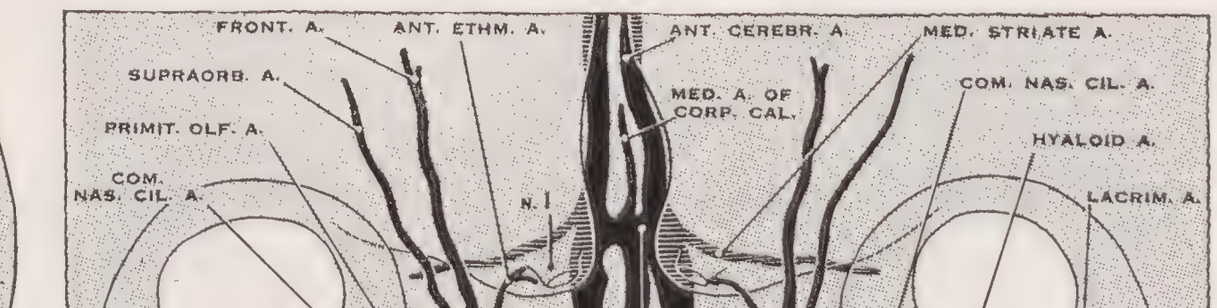
CRAN. DIVIS. (PRIMIT. OLF. A.)
OF
INT. CAROT. A.
PRIMIT. MAX. A.
MAND. A.
VENT. PHARYNG. A.
HYOID A.
N. VII
N. IX
AORT. ARCH
3
4
N. X
PHARYNG. BARS, 1 (MAX.) (MAND.)
POUCH 1
2 (HYOID)
POUCH 2
3
POUCH 3
" 4
PAIRED AORT.
Rathke
30
PRIMIT. MAX. A.
LING. N.
CHORD. TYMP. N.
TYMP. N.
VENT. PHARYNG. A.
N. VII
HYOID A.
N. IX
N. X
AORT. ARCH-PULM., 4, 3.
31
ANT. CHOROID. A.
PRIMIT. VENT. OPHTH. A. DORS. " "
PRIMIT. OLF. A.
NAS. FOSSA
REMNANT OF MAND. A. (VIDIAN A.)
SUPFIC. PETROS. N.
MAND. BAR
STEM OF STAPED. A.
POUCH 1
HYOID BAR
POUCH 2
COMPONENT OF EXT. CAROT. A.
N. I
INT. CAROT. A.
Rath.
STAPES
N. V
LING. N.
REMNANT OF VENT. PHARYNG. A.
HYOID A.
CHORD. TYMP. N.
N. VII
INNOM. A.
TYMP. N.
N. X
N. IX
AORT. ARCH
COM. CAROT. A.
DUCT. ART.
32


PRIMIT. OLF. A.
MID CEREBR. A.
PRIMIT VENT. OPHTH. A.
N. I
N. II
N. V (MAX.) (MAND.)
Rathke
PRIMIT. MAX. A.
VIDIAN A.
INT. CAROT. A.
ANT. CEREBR. A.
ANT. CHOROID. A.
SUPRAORB. N. AND A.
FRONT. N. AND A.
NASOCIL. N. AND ANT. ETHM. A.
COM. NAS. CIL. A.
HYALOID A.
COM. TEMP. CIL. A.
SECOND. ANAST. BETWEEN STEM OF OPHTH. A. AND SUPRAORB. DIV OF STAPED. A.
MAX. - MAND. DIV. OF STAPED. A. (MID. MENING. A.)
HYOID A. (CAROT. - TYMP. A.)
33
MED. A. OF CORP. CAL.
ANT. COMMUN. A.
MED. STRIATE A.
N. II
Hy-p.
MID. MENING. A.
INF. HYPOPH. A. (REMNANT OF PRIMIT. MAX. A.)
INT. MAX. A. (SECOND. ANAST.)
N. V (MAND.)
SUP. TYMP. A. (DWINDLING STEM OF STAPED. A.)
INT. CAROT. A.
STAPES PRIMORD.
35


PRIMIT. VENT OPHTH. A
CARTILAGE PRIMORD.
INT. CAR. A.
SECOND ANAST
COM. NAS. CIL. A.
" TEMP. " "
HYALOID A.
STEM OF OPHTH. A.


ANT. CEREBR. A.
PRIMIT. OLF. A.
NASOCIL. N. AND ANT. ETHM. A.
SUPRAORB. DIV. OF STAPED. A.
N. I
FRONT. N. AND A.
N. II
POST. COMMUN. A.
SECOND. ANAST.
Rathke
GANG. N. V
STEM OF OPHTH. A.
MAX. - MAND. DIV. OF STAPED. A.
INT. CAROT. A.
34


FRONT. A.
ANT. ETHM. A.
ANT. CEREBR. A.
MED. STRIATE A.
SUPRAORB. A.
MED. A. OF CORP. CAL.
COM. NAS. CIL. A.
PRIMIT. OLF. A.
HYALOID A.
LACRIM. A.
N. I
ANT. COMMUN. A.
SECOND. ANAST.
N. II
COM. TEMP. CIL. A.
POST. COMMUN. A.
Hyp.
SUPRAORB. DIV. OF
N. V
MAX. - MAND. DIV. OF STAPED. A. (MID. MENING. A.)
STEM OF POST. CEREBR. A.
BAS. A.
INT. CAROT. A.
36

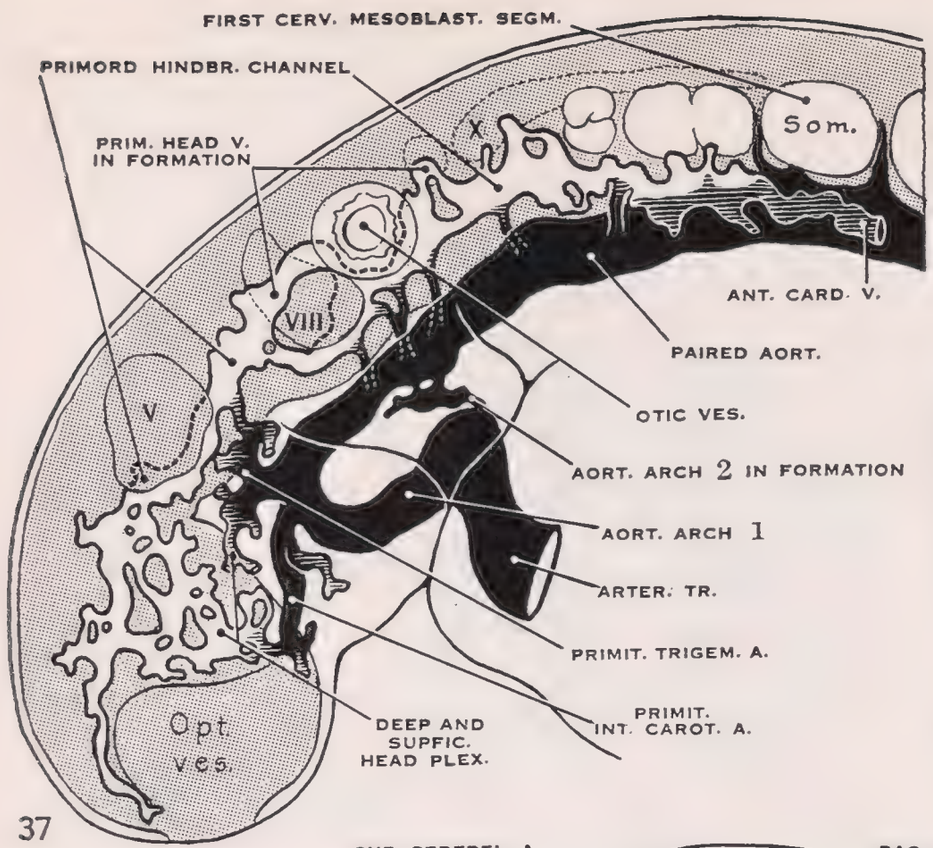
## Plate 4

Lateral and horizontal (ventral view) reconstructions of four embryos to illustrate the formation and significance of the primitive trigeminal artery. (See also text figs. 1, 3; and pl. 5.)

Fig. 37. (No. 2053, 3 mm., 20 somites. See coronal view in fig. 43, pl. 5.) This is the youngest embryo of the present series and illustrates the most primitive vascular system of the head. The primordial hindbrain channel (shown white because it serves temporarily as a vein), which has already developed fragments of the primary head vein on either side of the otic vesicle, is fed by aortic sprouts concentrated in the trigeminal and otic regions. The first aortic arch gives off the primitive internal carotid and the primitive trigeminal artery, which is the most constant and conspicuous of the temporary presegmental branches of the paired aorta. ×60.

Figs. 38, 39. (Lateral and ventral views of no. 836, 4 mm.) At this stage the third aortic arch has formed, and the primordial hindbrain channel has disappeared during the evolution of a recognizable system of cranial arteries and veins (note that the primary head vein, shown white, is fully formed). The primitive internal carotid supplies the optic vesicle, forebrain, and midbrain. Plexiform fragments of a vascular tract, representing the future bilateral longitudinal neural artery, lie on the hindbrain wall and are fed by aortic presegmental branches in the trigeminal, otic, and hypoglossal regions. Since the first aortic arch has been replaced by the succeeding mandibular artery, that part of the aorta giving origin to the trigeminal artery is now considered a part of the internal carotid. In a succeeding stage, shown in text figure 1, the bilateral longitudinal neural artery is fully formed; its cranial end is supplied by a large trigeminal artery and its caudal end by the first cervical segmental artery, reinforced by the otic and hypoglossal arteries. The early obliteration of the primitive otic vessel is, in part, dependent upon the consolidation of the neurals in the mid-line to form the basilar artery, and their coincident withdrawal from the carotid, which arches laterally in this region (text figs. 1, 2). ×33.3.

Figs. 40, 41 (lateral and ventral views of no. 544, 11.5 mm.), and 42 (no. 940, 14 mm.; see lateral view in text fig. 6). Late persistence of the primitive trigeminal artery as a large caroticobasilar anastomosis in two embryos of the early postbranchial period. As shown in text figures 2 and 3, the trigeminal artery usually disappears at an earlier stage, and as soon as its role of cranial supply to the bilateral neural artery (or basilar artery, formed by the neurals) is taken over by the definitive posterior communicating artery. In the atypical cases shown here, it is to be noted that the caudal end of the posterior communicating artery is small at its junction with the basilar artery, which is still being supplied in large part by the trigeminal branch of the carotid. On the right side of the embryo in figure 41, however, where the trigeminal artery is only indirectly connected with the caudal end of the basilar, the posterior communicating artery is characteristically large throughout its whole length. (Carotid and posterior communicating arteries shown in white to permit view of arteries beneath.) ×15.

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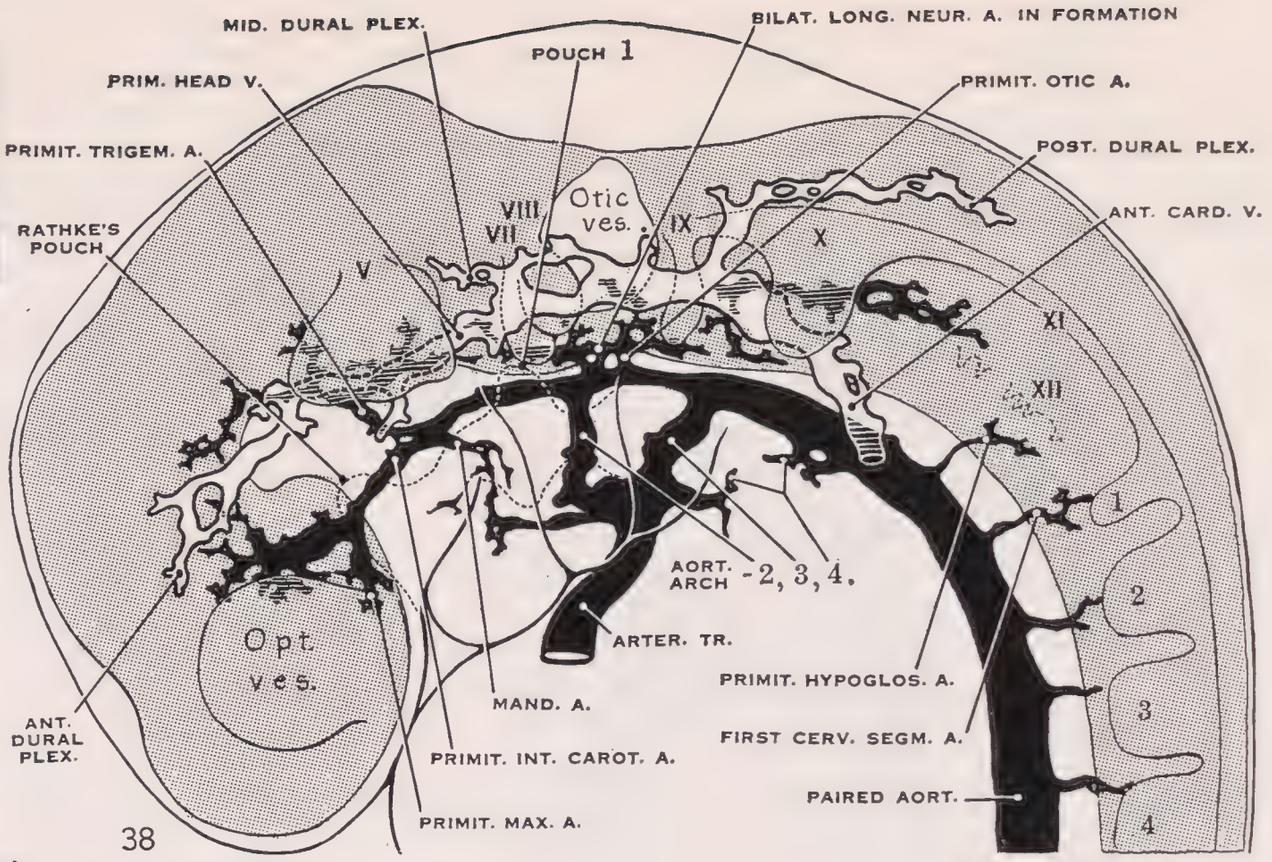


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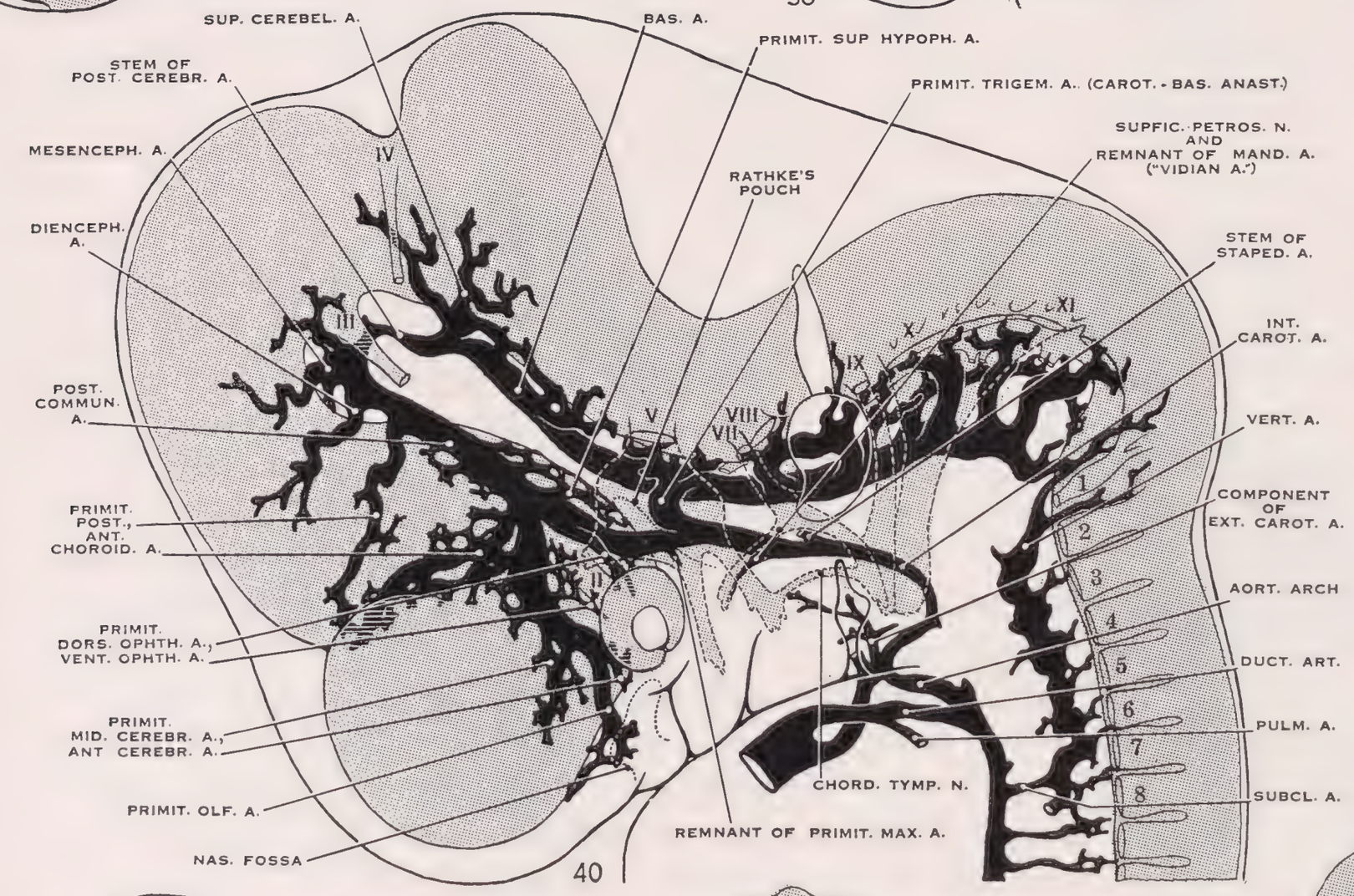


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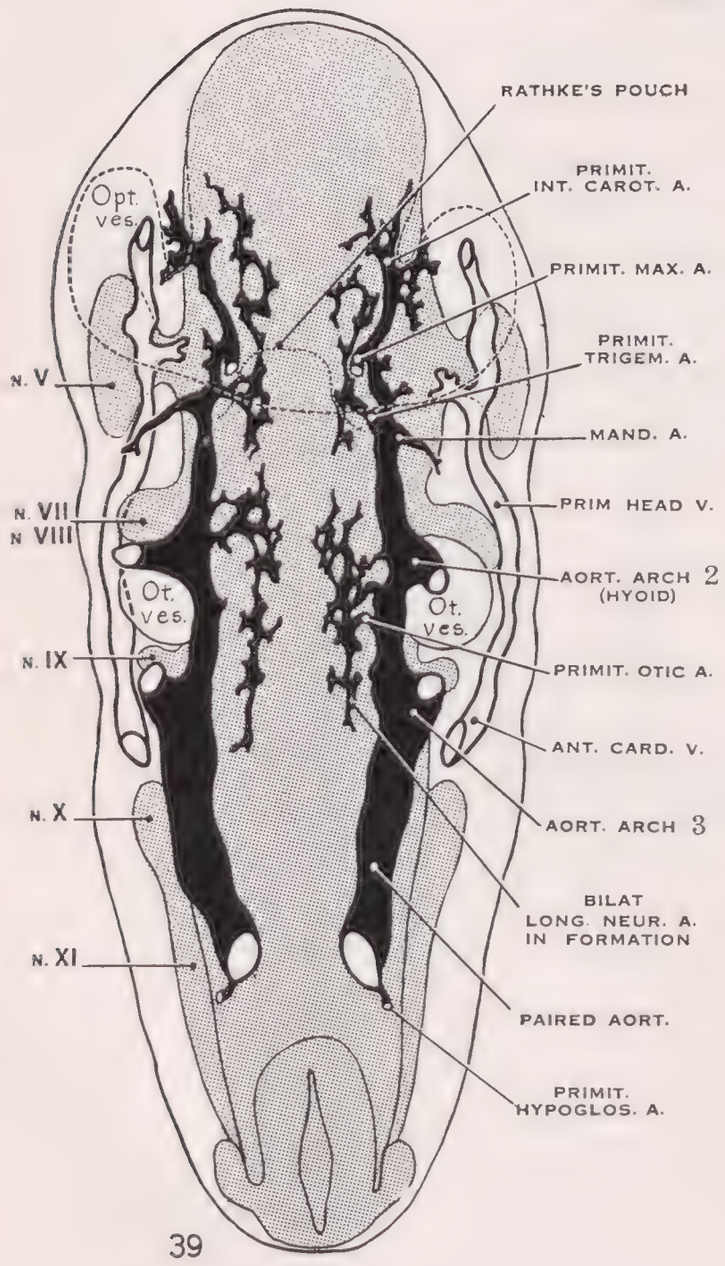


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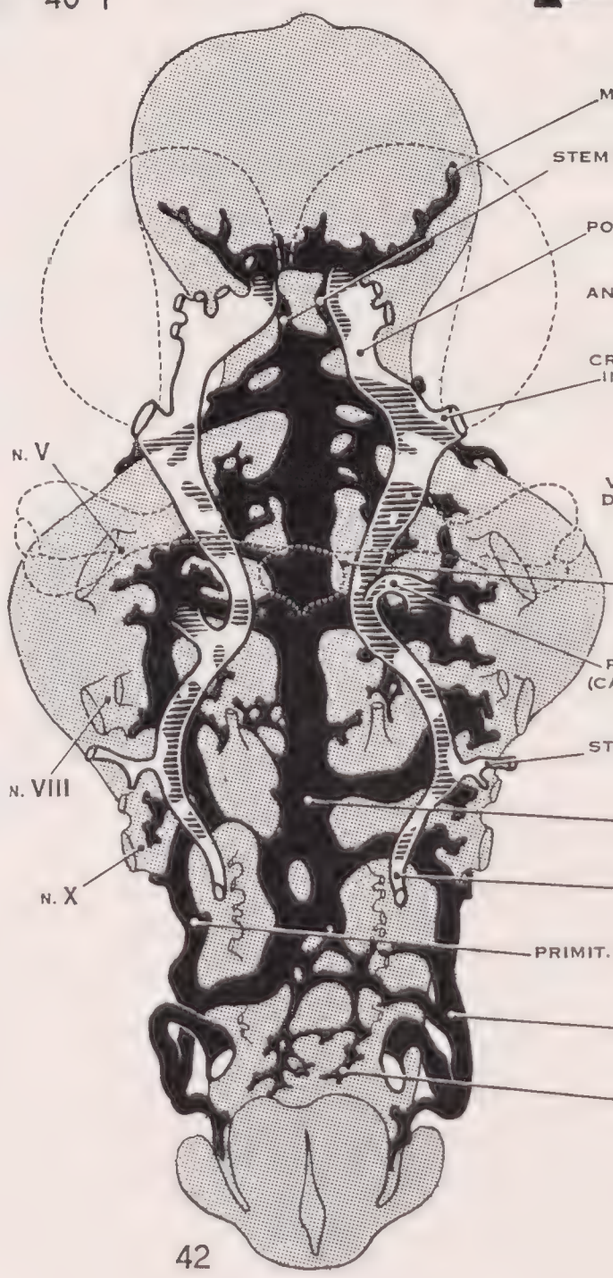


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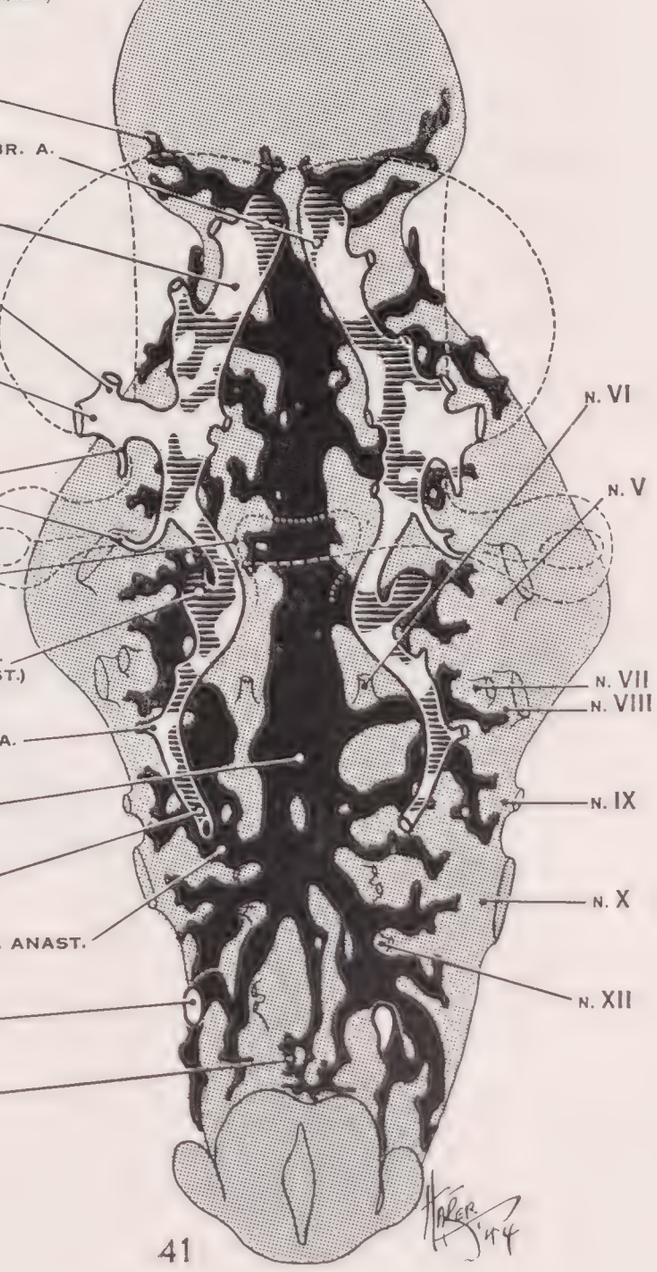


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## Plate 5

Partial coronal reconstructions of six embryos from 3 to 43 mm., illustrating the origin and fate of the temporary trigeminal artery. Each is viewed from behind, as if sectioned at the level of the hypophyseal diverticulum and fifth nerve (excepting the first embryo, which is viewed just behind the eighth nerve), so that the right side of the picture shows the right side of the embryo.

Fig. 43. (No. 2053, 3 mm., 20 somites. See lateral view in fig. 37, pl. 4.) The primitive internal carotid, in its present origin from the paired aorta at the first aortic arch and in its course, is mesial in contrast with the primitive trigeminal artery, which arches laterally, supplying the head plexus en route to its termination in the primordial hindbrain channel. ×60.

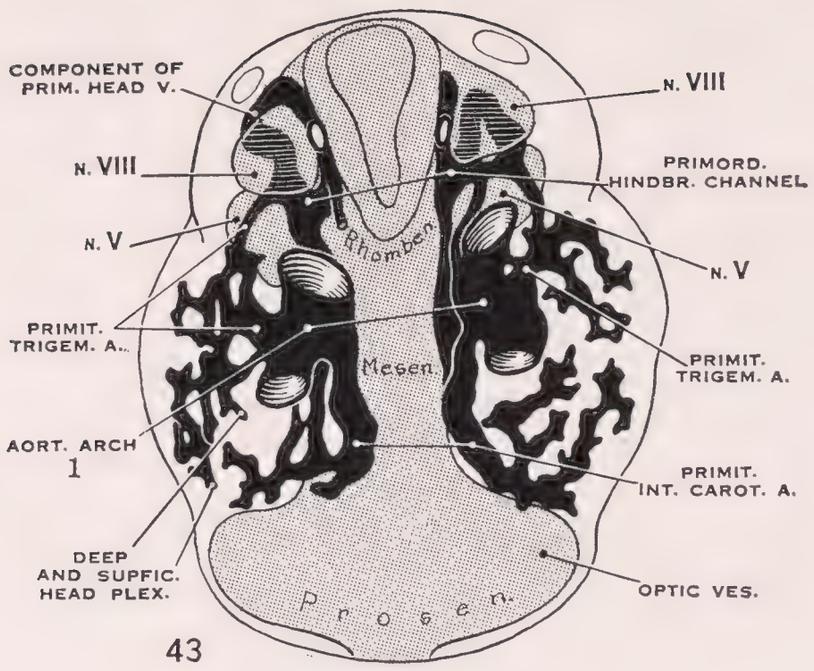
Fig. 44. (No. 588, 4 mm. See lateral and ventral views in text fig. 1.) The bilateral longitudinal neurals which precede the basilar artery are an end result of differentiation of the primordial hindbrain channel. The paired aorta of the preceding embryo (fig. 43) is now the internal carotid artery, following the involution of the first two aortic arches. It divides at Rathke's pouch into two branches: one is the prolongation of the carotid to the forebrain; the other is the temporary trigeminal artery, which supplies the cranial end of the longitudinal neural artery. Note that the posterior communicating artery is not yet a definitive continuous channel. ×40.

Fig. 45. (No. 1121, 11.8 mm.) The completed posterior communicating artery has usurped the role of the primitive trigeminal artery in supplying the cranial end of the basilar artery, which has formed through consolidation of the bilateral neural arteries. Note the remnants of the interrupted trigeminal artery, of which both dorsal and ventral ends persist on the left side of the embryo. ×15.

Fig. 46. (No. 940, 14 mm. See lateral view in text fig. 6; ventral view in fig. 42, pl. 4.) Late persistence of the trigeminal artery as a large caroticobasilar anastomosis after completion of the posterior communicating artery, which, as a result, is unusually attenuated at its caudal junction with the basilar artery (see other views of this embryo noted above). ×15.

Fig. 47. (No. 966, 23 mm. See coronal or face view of head in text fig. 9.) At this stage the trigeminal artery is sometimes represented by a small plexiform channel connecting the carotid and basilar arteries at the hypophyseal level. On the right side of this embryo, a basilar remnant of the artery extends as far as the primitive fold of dura which later forms part of the sheath enveloping the Gasserian ganglion. ×15.

Fig. 48. (No. 886, 43 mm. See lateral and ventral views in text fig. 10.) In the oldest embryo of this series, there are also bilateral remnants of the trigeminal artery. These terminate at the condensation of mesenchyme extending laterally from the primordial sphenoid cartilage, and presumably persist in the adult as branches of the basilar artery to the region of the fifth nerve. ×6.

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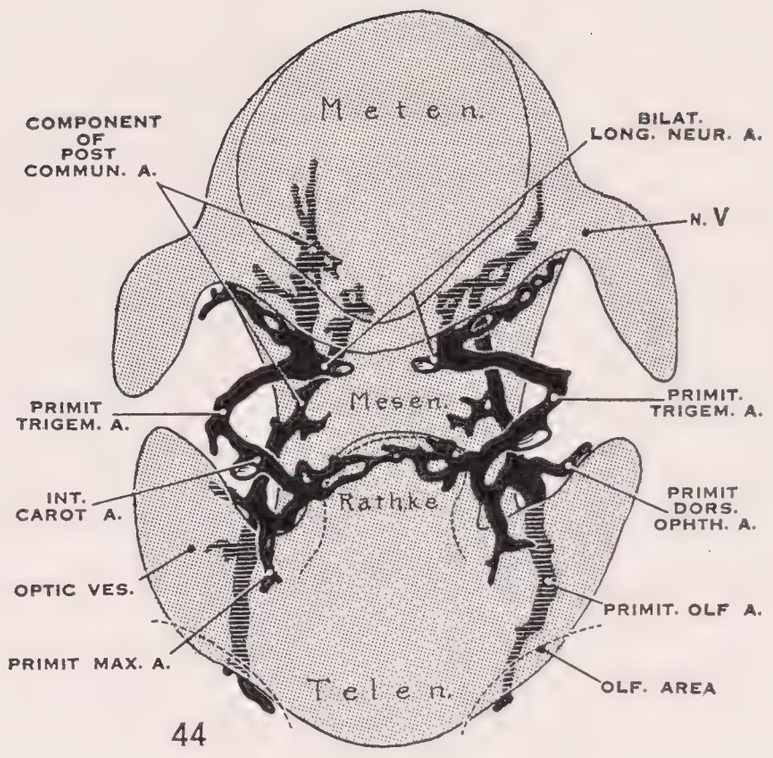


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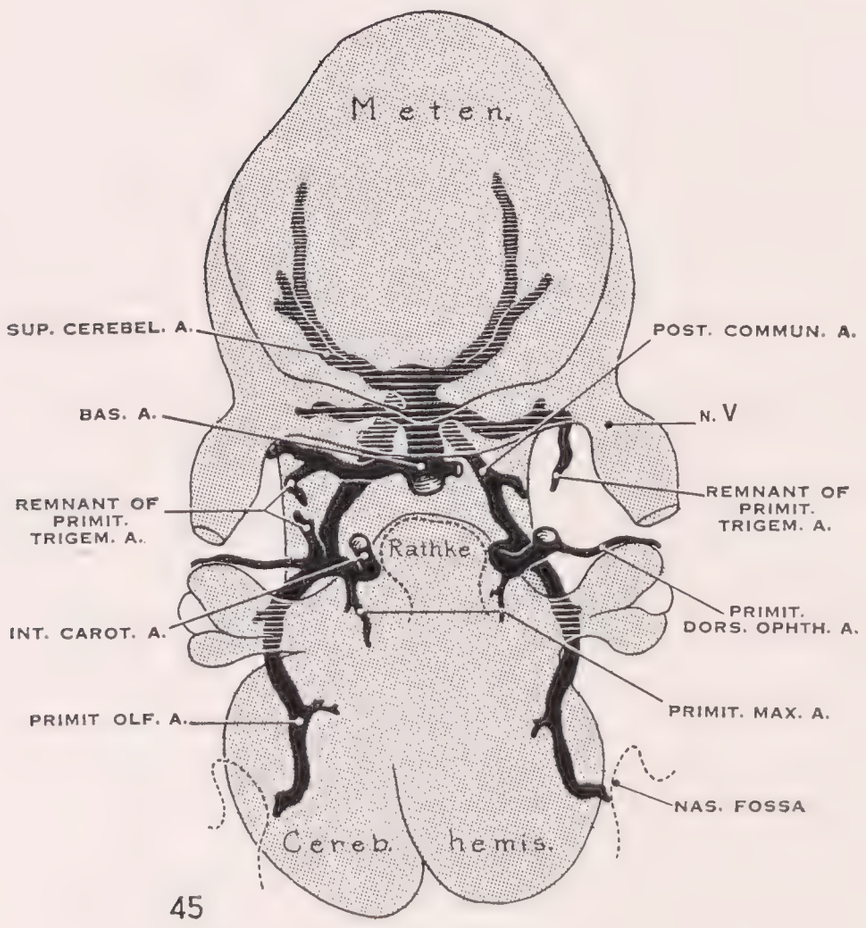


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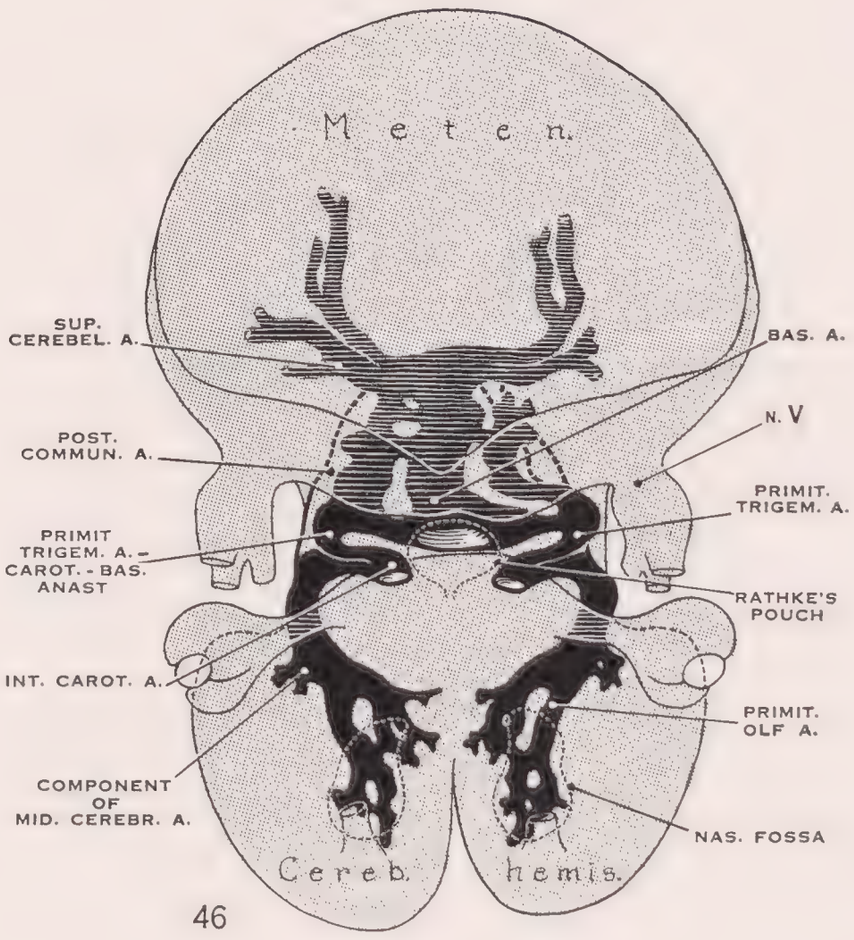


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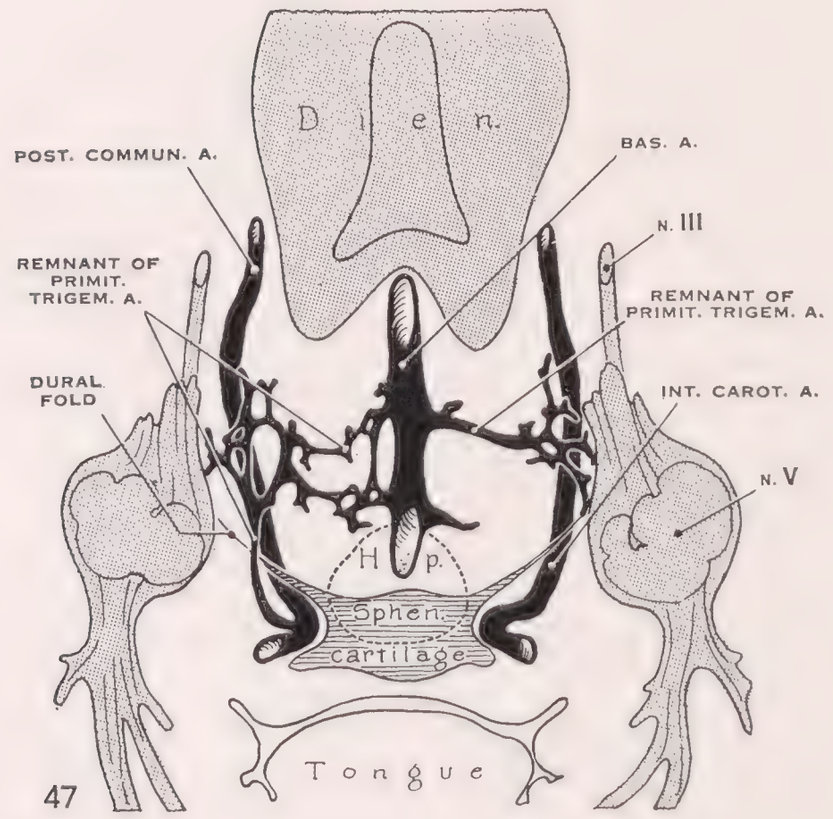


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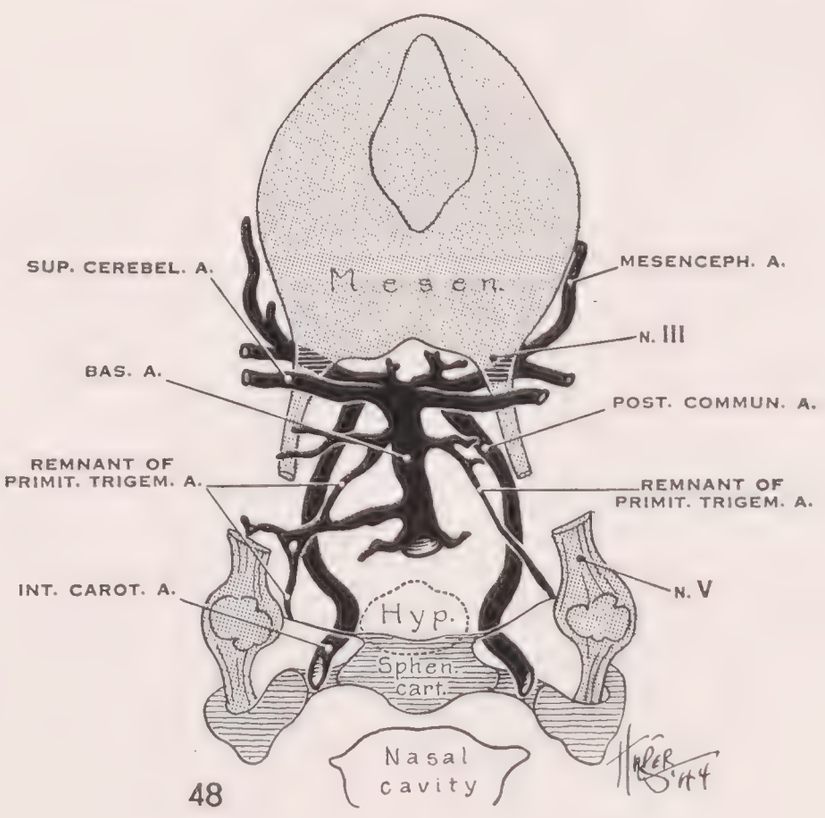


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